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# THE FOOT OF RIELLA AMERICANA AND ITS RELATION TO NUTRITION OF THE SPOROPHYTE

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(WITH SIXTEEN FIGURES)

## Introduction

Of the three parts of the sporophytic generation in the bryophytes, the foot and seta have received much less attention than the sporange. Once almost universally considered to function both as an anchoring and as a necessary absorbing organ, the foot has recently been re-examined, both as to structure and function, particularly in connection with the frequent partial nutritional independence of the sporophyte from the gametophyte. This paper presents an account of its developmental stages, confirms evidence of sporophytic independence, and adds some anatomical considerations which suggest a possible reversal of the usually accepted parasitic relations between the two generations.

The fullest anatomical accounts of the foot in liverworts are found in papers by LEITGEB (**19**) and MEYER (**22, 23, 24**), with summaries by GOEBEL (**13**). For the moss foot, the most complete papers are those of LORCH (**20, 21**) and BLAIKLEY (**2**). For the several species of the aquatic liverwort *Riella*, the foot has received scarcely more than a mention from LEITGEB (**19**), PORSILD (**26**), and CAVERS (**9**), and developmental stages have apparently not been studied. HOWE and UNDERWOOD (**15**), describing *R. americana*, do not mention the foot, and none of the illustrations shows details of its tissues. Up to the present there has been no description of the mature foot and no account of the ontogeny of this organ for the species just mentioned.

In nearly all liverworts and mosses the foot originates by division of cells at the base of a more or less elongated seta. Its shape at maturity varies, but it is gen-

erally a smoothly rounded or oval structure imbedded more or less deeply in the gametophytic tissues at the base of the calyptra.

Deviations from such a smoothly rounded pattern have been known since 1874, when KIENITZ-GERLOFF (16) showed that the peripheral cells of the foot of *Frullania* protrude slightly into the gametophytic tissue. LEITGEB (19) found a similar papillate condition in *Frullania*, *Aneura*, *Mörkia*, *Riella helicophylla*, and *R. parisii;* in *R. helicophylla* some of the cells are elongated and extend from middle to periphery of the foot. Much longer protruding processes were discovered (19) in the foot of *Anthoceros* and *Dendroceros*. Still longer penetration filaments were reported by GOEBEL (11) for the moss *Diphyscium*, and later (12) for *Eriopus*, where they originate on the seta instead of on the foot. Superficial foot cells vary in the different genera from smooth or slightly elevated to very long, branched filamentous structures. Many nouns and descriptive adjectives have been applied to these extensions, including papilla, papillate, papillose, haustorium, haustorial outgrowth, root hair, haustorial rhizoid, haustorial tube, rhizoid-like tubule, outgrowth, tubular outgrowth, process, protrusion, appendage, and filament. As a substitute for most of these terms, the following are proposed in the hope that they will confer a greater relative value. Peripheral cells which have the superficial contour of the foot are called smooth. The term gibbous is applied to those which are rounded at the margin of the foot. For longer penetrating structures the terms subdigitate and digitate are proposed, and for structures long enough to resemble rhizoids or root hairs, the term filament. Throughout, the noun process or protrusion is suggested for the protruding cell or portion of a cell, when this is longer than that represented by the term gibbous. Common descriptive adjectives are also needed, as forked, branched, and irregularly branched.

In addition to the papers already cited, gibbous cells or processes of various degrees of extension have been described or illustrated in the foot by PORSILD (26) for *Riella paulsenii*, LANG (18) for *Notothylas*, O'KEEFE (25) for *Targionia*, LORCH (21) for mosses as *Bryum*, BLAIKLEY (2) for a number of mosses and *Anthoceros*, and VRABER (28) for *Riella helicophylla*. Often these were merely simple observations, or even only diagrams without mention in the text. The most common interpretation, when any was given, has been that the processes serve as absorbing organs (13). BARTLETT (1), however, considered that many of the protrusions attributed to the foot in *Anthoceros* belong in reality to the gametophyte. BUCH (6) accepted the similarity, both in structure and function, of the processes in the foot of the Anthocerotales to the rhizoid of the gametophyte. BLAIKLEY noted that, in general, those mosses in which the contact between the two generations is the closest are the most likely to have their peripheral foot cells extended into processes. BOWER (5) observed that the processes of the foot increase its absorbing surface and extend its range of absorption.

Enlarged cells in the foot have been found by CAMPBELL (7) in *Geothallus*. Enlarged nuclei are described in the same genus (7), as well as in *Eriopus*, by GOEBEL (12), in *Plagiochasma* by MEYER (23), and in the mosses by BLAIKLEY (2). Abundant, or a dense, deeply staining protoplasm was seen in *Geothallus* by CAMPBELL (7), in *Eriopus* by GOEBEL (12), in *Corsinia* and *Plagiochasma* by MEYER (22, 23), in *Targionia* by O'KEEFE (25), and in mosses by BLAIKLEY (2). Thickened or otherwise modified cell walls were reported by GYÖRFFY (14) for *Molendoa*, and by LORCH (20) and BLAIKLEY (2) for various mosses. Modifications in the gametophytic cells adjacent to the foot were noted in *Corsinia* by MEYER (22), in *Targionia* by O'KEEFE (25), in *Anthoceros* by CAMPBELL (8), and in several mosses by BLAIKLEY (2).

That the sporophyte in both liverworts and mosses has a completely dependent nutritional existence, except in the Anthocerotales, is still rather commonly accepted, as seen in quotations cited by BOLD (3). CAMPBELL (7), in fact, interpreted abundant cytoplasm and large nuclei as an indication that the foot cells were actively engaged in the nutrition of the sporophyte of *Geothallus*. GOEBEL (12) considered that the processes of the seta of *Eriopus* transferred food materials from both foot and vaginula into the seta and thence into the sporange. As early as 1908, however, DOUIN (10) noted the presence of chloroplasts in all cells of the foot of *Sphaerocarpus*, the closest relative of *Riella*. MEYER (23) believed in the heterotrophic nutrition of the young sporophyte of *Plagiochasma*, but in at least partial nutritional independence in later stages, since chloroplasts and glycose were found in foot and sporange. The same writer (24) found the foot cells and sporange wall cells of *Corsinia* to have a green color. In 1924, CAMPBELL (8) kept an excised sporophyte of *Anthoceros* alive for 3 months and discussed its nutritional independence. GOEBEL (13) recognized the probable independence of the sporophyte of some of the bryophytes. BOWER (5) stated that the sporophyte of *Buxbaumia*, which also possesses processes in the foot cells, becomes nutritionally independent in its more mature stages. STUDHALTER (27) demonstrated complete nutritional independence during the latter half of the development of the sporophyte of *Riella americana* and *Sphaerocarpus texana* by growing excised sporanges to maturity in tap-water cultures; these not only reached their full term, but they ripened their spores, which in turn germinated normally and abundantly. BOLD (3, 4) found chloroplasts and included starch in the foot, seta, and capsule of various liverworts and mosses; at least partial nutritional independence was indicated for the sporophyte.

## Material and methods

The plants upon which this study is based were collected in the Davis Mountains of Texas or else grown in laboratory cultures. Fixing was done in formalin-

acetic-alcohol, and staining with safranin or Delafield's haematoxylin or with various combinations. The usual paraffin method was used. Both stained and unstained whole mounts were studied for comparison.

## Observations

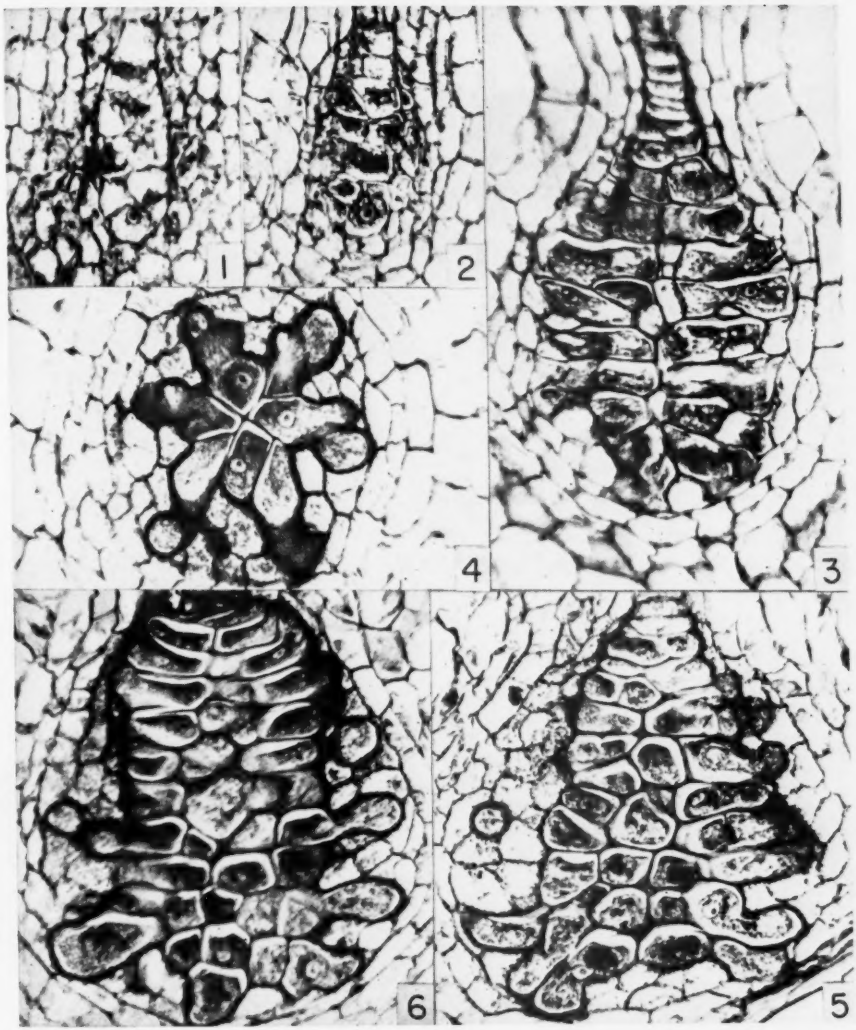
### Origin and early growth of the foot

In the eight-celled stage, the sporophyte is divided by oblique walls in such a manner that a single cell points downward into the tissues of the subcalyptral pad. From this cell soon originates a row of four to eight short cylindrical cells which, by the upward growth of the gametophytic tissues, come to be deeply imbedded in the latter. The walls of these cells are uniformly thin, and the cross walls are perpendicular to the axis; cell divisions have taken place in a single plane.

The basal portion of this young seta broadens slightly, and further cell divisions in the basal region ensue in new planes, resulting in several oblique cross walls (fig. 1). This stage, the first definitely to be recognizable as a foot, makes its appearance when the sporange has a diameter of seven or eight cells, of which the outermost layer has been differentiated into a relatively thick sporange wall. The cells of the very young foot are isodiametric, angular toward the interior of the foot, rounded at its periphery, and of the same size as most of the cells of the subcalyptral pad. Their cell walls are uniformly thin, not darkened, and stain much as do the walls of the cells of the calyptra and involucre. The nucleus is large and prominent and usually occupies a central position; it is oval or spherical, and has a diameter range of 8–13 $\mu$, with an average of about $7.5 \times 10$ $\mu$. There is a single spherical nucleolus which measures about 5 $\mu$ in diameter. It is surrounded by a clear zone extending out to the thin but prominent nuclear membrane. These sporophytic nuclei remain the same size throughout the development of the foot and are much larger than those of the calyptra and involucre, which average 5–7 $\mu$ in diameter. The cytoplasm is meager in amount and occupies a peripheral position. When the nucleus is centrally located, a thin layer of cytoplasm surrounds it and several thin cytoplasmic strands connect with the peripheral cytoplasm. In early stages, therefore, there is a large, clear central vacuole.

The zone of contact between the foot and the calyptra is nearly always intimate, there being no evidence either of mucilage or of intercellular spaces. The latter are also lacking within the foot.

The first oblique and irregular divisions are soon followed by more regular ones, involving vertical walls near the median line of the foot (fig. 2). As a result, the cells at this stage tend to lie at the same level in pairs, and a pair extends completely through the center of the foot from one margin to the opposite one. The median cross walls are not all in the same plane. In a young foot five cells long and definitely only two cells wide, the sequence from top to bottom (in which wall 1 at




FIGS. 1–6.—Fig. 1, oblique longisection of young foot showing its differentiation from lower end of seta. Fig. 2, longisection just off median line showing increase in mass of cytoplasm, beginning of plasmolysis, and binucleate cell. Fig. 3, longisection of foot with gibbous to subdigitate, binucleate, radially elongated cells; cytoplasm becoming coarsely granular. Fig. 4, cross-section through middle of foot with digitate, radially arranged giant cells, walls of variable thickness, and discontinuous overlapping wall near center. Fig. 5, longisection of foot with gibbous and digitate cells, one of latter (at left) bent at right angles and cut in cross-section. Fig. 6, longisection of foot shown in fig. 7, just off median line, showing thickened walls, discontinuous and overlapping at two points in upper portion. ×270.

the top of the foot is used as a basis for orientation in a circle) was as follows: wall 2, 30° to the right of wall 1; wall 3, 60° to the right of wall 1; wall 4, 30° to the right of the first wall; and wall 5, again 60° to the right of wall 1. At any given level, four cells often appear to radiate from the center, 60°–90° apart. Occasionally there appear to be six cells around the central point, instead of four.

There is naturally a tendency for a cell to be narrow at the middle of the foot and to broaden tangentially toward the periphery. Many cells are broadly triangular in cross-section of the foot. The margin of the foot remains for a time essentially smooth, and the entire foot has the shape of a much elongated oval. Cells measure 20–25 × 25–40 μ at this stage, while the entire foot measures 30–50 × 40–80 μ. Plasmolysis is sometimes pronounced.

### Maturing foot

Profound changes in the structure of the foot usually occur about the time this organ is eight to ten cells long (fig. 3). Simultaneously, the seta has a length of ten to twelve cells, and the sporange, which has not yet differentiated spore mother cells and nurse cells, has a diameter of about ten cells.

Growth in size of the foot proceeds steadily, until at maturity a length of nine to twelve cells or 200–250 μ or more is reached and a width of 150–200 μ. In shape the foot is broadly ovate. Figure 7 shows the relations between sporange, seta, foot, calyptra, involucre, and subcalyptral pad in a sporophyte in which nurse cells and spore mother cells had become differentiated.

Perhaps the most obvious changes in the maturing foot are those associated with the shape of the constituent cells. At first broadly triangular in outline, they gradually change into many different shapes. With increase in the diameter of the foot, the cells elongate radially (fig. 3). Remaining narrow at the center, they usually broaden materially toward the margin, especially as seen in cross-section (fig. 4). Since the cells are so compacted that intercellular spaces are virtually absent, some of them grow in various directions and attain many grotesque shapes, in both cross- and longisection. Occasionally long, very slender cells are found extending from center to margin (figs. 5, 14). At the base the cells abandon their truly radial direction of growth and extend downward in lines more nearly parallel with the axis of the foot (figs. 3, 5). Many cells, even at later stages, extend from the median line of the foot outward to its margin (figs. 9, 10, 12, 13). Cell diameters vary greatly, no matter in which direction a section has been cut. Often with increasing age cross walls are formed which divide the radially elongated cells into two or three shorter ones. The fundamental plan of cells extending radially is found in all parts of the foot.

The arrangement of four cells radiating from the center about 90° apart is often

in evidence (fig. 4), even toward maturity of the foot. More frequently, however, the plan is actually or apparently obliterated, so that four to seven cells appear to radiate from near a common point.

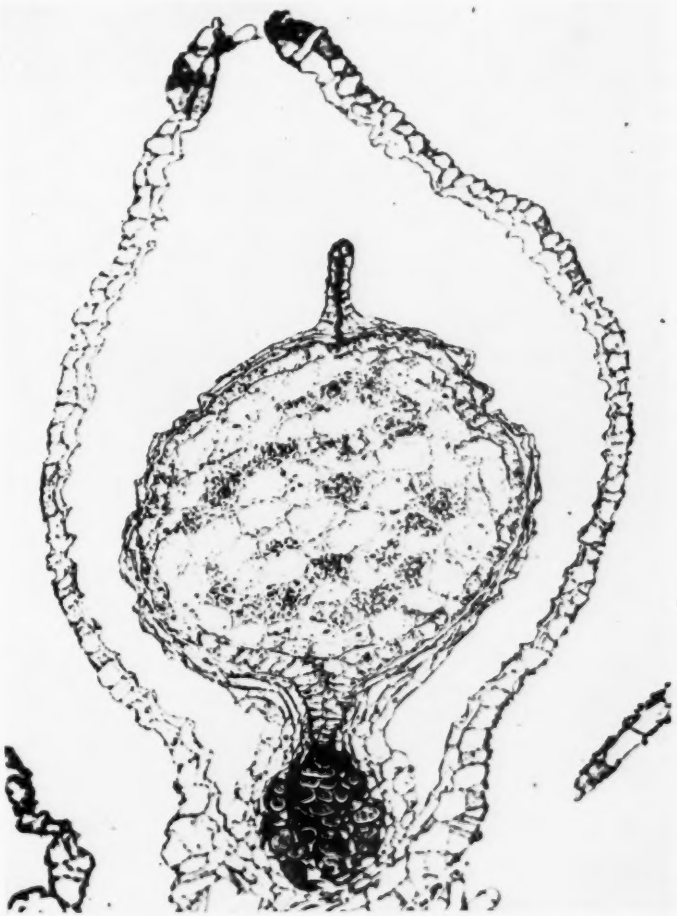


FIG. 7.—Longisection through involucre and its ostiole, calyptra, archegonial neck, sporange, seta, and foot, the latter cut slightly to one side of median plane. ×67.

It is at the margin of the foot that the cells assume their most grotesque shapes. Many become bent, twisted, or deeply lobed or forked into equal or unequal parts, with the free tips varying from angular to very broadly rounded (figs. 9, 10, 12, 13). Individual cells frequently become gibbous and remain so to their maturity

(fig. 5). Others extend farther out as subdigitate processes, which penetrate more or less deeply among the cells of the subcalyptral pad (fig. 3). Still others are elongated into truly digitate processes, which protrude deeply into the gametophyte (figs. 4, 5, 6, 12, 14). The latter may be straight, twisted, or sharply bent, sometimes even at right angles; in a longisection of a foot it sometimes happens that some of them are cut lengthwise, while others, which had grown at right angles, are cut in perfect cross-section (fig. 5, left). A longisection or an oblique one through the margin of the foot often cuts through no other part of this organ than the digitate processes, each of which has the appearance of a circular, thick-walled cell (fig. 8). In cross-section such a process may be 42 $\mu$ in diameter. Digitate protrusions are more numerous in the lower half or two-thirds of the foot. All types of processes classified in the introduction as having been described in certain other liverworts and mosses have been found in *Riella americana*, with the exception of filaments.

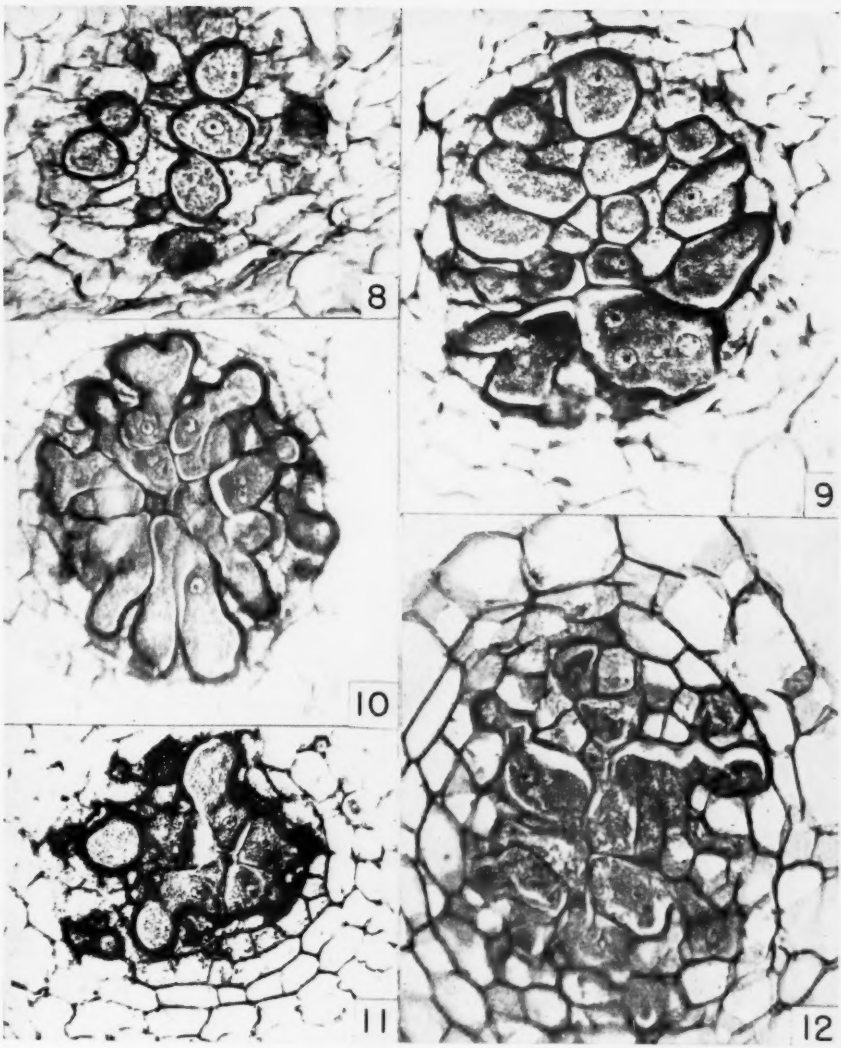
The actual three-dimensional shape of the foot cells ranges from the one extreme of rather simple boxlike structures to the other of irregularity which defies description. In general, however, the longest dimension lies radially in the foot, and the cells are broader in cross- than in longisection of this organ.

There is also great variation in the size of the cells. Some of the smaller ones are little larger than those of the surrounding gametophyte (fig. 9). A large proportion of them are giants, however, not only in length but often in breadth as well (figs. 4, 9, 10, 11, 12, 13). Since they often extend half way through the foot, it is not surprising to find them measuring 100 $\mu$ in length; a few reach at least 110 $\mu$. Some of the wider unbranched ones measure up to 70 $\mu$ in width. A long narrow cell was found to measure $20 \times 100$ $\mu$, as seen in one section. Lobed and forked cells may be as wide as 70 $\mu$.

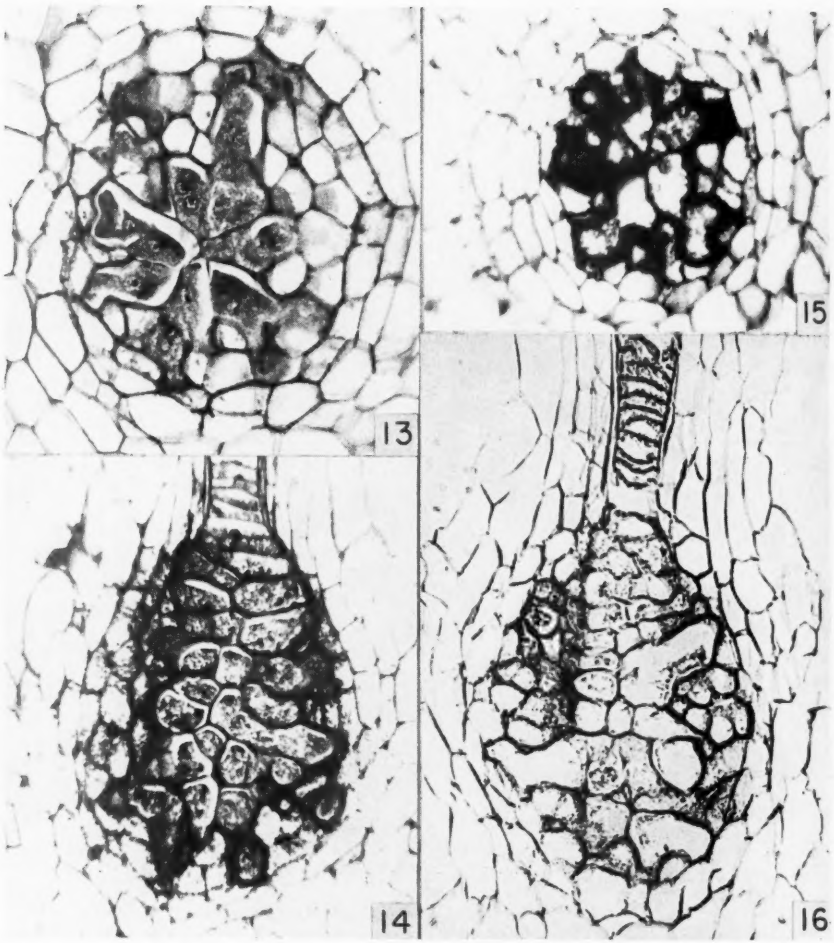
It is difficult to estimate the total number of cells in the mature foot. At maturity this organ is ten to twelve cells long. Assuming that the majority of cells extend from middle to periphery, there should be forty to sixty cells in all. But since some of them are further divided radially, the total number is probably much greater.

With such variation in cell shape, it is not surprising that the margin of the foot is usually very irregular. Although the general shape is oval, the actual zone of contact between foot and gametophyte is usually a very irregular line. This boundary is generally a definite one (figs. 4, 7); at other times it is not clearly defined, since those cells of the latter which are adjacent to the sporophyte (one to three rows) occasionally become somewhat modified (fig. 14). The contact between foot and gametophyte, however, is almost without exception intimate throughout its development, lacking both mucilage and intercellular spaces.

The first change to take place in the cell wall is one of thickening and darkening.




FIGS. 8–12.—Fig. 8, longisection of extreme edge of foot shown in fig. 7, consisting of cross-section through several digitate processes, one showing nucleus. Fig. 9, oblique longisection through side of foot showing forked cell, giant cell with four nuclei (nucleoli of two of them present in adjacent section), and long irregular cell just above the latter. Fig. 10, cross-section through middle of foot with digitate, forked, and irregular giant cells, cleaved cytoplasm in lower forked cell, and much variation in wall thickness. Fig. 11, oblique section through upper part of foot of small sporophyte approaching maturity, with variable wall thicknesses. Fig. 12, cross-section below middle of foot with giant branched cell at middle right which shows two parallel partial walls, at free end of one of which lie two nuclei at different optical levels (only one shows in photograph). ×270.




FIGS. 13–16.—Fig. 13, cross-section farther below middle of same foot as in fig. 12, showing granular degeneration of nuclei and cytoplasm, and partial wall in cell at left. Fig. 14, longisection, almost median, of mature foot showing granular degeneration of protoplasm of foot cells and some thick-walled granular cells in adjacent gametophyte. Fig. 15, cross-section just below middle of foot of dwarf mature sporophyte showing thick walls and degenerated protoplasm. Fig. 16, longisection of very old foot in last stages of granular degeneration, with varied walls. ×270.

The former change, which at first proceeds at a uniform rate over the entire cell, soon becomes irregular and results in much variation in thickness in different parts of the foot (figs. 4, 10, 13), in a single cell (figs. 10, lower; 13, left), or even in a single short wall (fig. 13, lower middle). Extreme cases of variation in different parts of the foot are shown in figures 11 and 15. Although one of these is a cross-section and the other oblique, it is probable that all the thickest walls shown have been cut obliquely; they measure 4–5 $\mu$ in width, which may be interpreted as 3–4 $\mu$ in actuality. Cell walls appear commonly thicker at the periphery than in the interior of the foot; this is due only in part to a larger percentage of oblique cuts through the rounded marginal portions of the cells. A great deal of variation is also found in a single wall, which may taper rapidly from very thick to quite thin within a short length.

The color of the wall varies from nearly colorless in the younger foot, progressively through deeper shades, to a very dark black. It is never yellow, which is the color assumed by the mature seta. Staining does not appear to have an appreciable effect on the shade of color exhibited in the darker walls.

In the young foot all walls are typically continuous, although some of them may be folded, as in the crotch of a lobed or forked cell. Frequently in older cells the wall is interrupted; it may have the appearance of having been broken, with one or both ends displaced laterally (figs. 5, 16). At other times the ends have become altered so that they do not appear any longer to fit together. The probable cause of such interruption is often a simple breaking at a weak point in the wall; often, however, a portion of the wall seems to have been absorbed. At other times the walls are both discontinuous and overlapping, with each end frequently tapering to a long point (figs. 4, 6). Partial walls are also found (figs. 12, right; 13, left). These extend at various angles toward the interior of a cell, sometimes only for a short distance, at others nearly to the opposite wall. Such a partial wall may be uniform in thickness, with a blunt or rounded end, or it may taper to a point. In rare instances a partial wall extends more deeply into the cell at one optical level than at another, as shown by its passing directly over the top of a nucleus (fig. 12, right). There is usually no discernible definite point on the opposite wall at which the partial wall had once connected with the former. It seems most likely that the partial wall is the result of partial absorption by the protoplasm. Perhaps some of the other wall irregularities are due to the same factor. Such absorption, of course, results in the coalescence of adjacent cells; but it should be emphasized that partial walls are not very common, that most walls are continuous, and that nearly all the giant cells have attained their size by enlargement.

Long before the foot is mature or the cells have reached their maximum size, a limited free nuclear division takes place. Two nuclei per cell (figs. 10, 13) are common, often so common that half the cells of a single section are binucleate. Three

nuclei are less common and four even less so. At times a single cell will show three or four nuclei in the same section (fig. 9). There is no definite proof of more than four nuclei in one cell. Binucleate cells are first found in a relatively young foot (fig. 2). The formation of binucleate cells by the dissolution of a cross wall and the fusion of the respective protoplasts is possible, but this occurs only at infrequent intervals. Likewise, the fusion of two binucleate cells would theoretically give rise to a cell with four nuclei. Since three or four nuclei are found in giant cells which lack partial walls, apparently free nuclear division is the more common cause of the multinucleate condition. Nuclei are the same size in uninucleate and multinucleate cells. The position of a nucleus is usually central; in a digitate process it is always located some distance back from the tip.

Just as nuclear division began in the relatively young foot, so also are several changes in the cytoplasm first seen at a very early stage in the development of this anchoring organ. The first noticeable change is a great increase in the mass of the cytoplasm (figs. 2, 3, 4), which soon occupies virtually the entire space within the cell wall. The large central vacuole becomes obliterated, to be replaced by numerous small irregular clear spaces in all parts of the cytoplasm. Shortly afterward these also disappear. With increasing age, the cytoplasm becomes progressively coarser in structure, the granules increasing materially in size (figs. 3 *ff.*). A single cell is not necessarily uniform in the type or degree of granulation (fig. 9, lower right). The color of the cytoplasm varies from its original colorless condition through darker shades to a deep black. Frequently different portions of the cytoplasm of a single cell vary appreciably in color and density; extremes of light gray and jet black may appear side by side in a single cell. Very dark cytoplasm is especially prominent in highly plasmolyzed cells.

Plasmolysis of the cytoplasm is common and begins early (fig. 2). In an older foot the cytoplasm may be drawn completely away from the wall, so that it occupies a central position. The shrinkage is not due to fixing or staining agents but to factors within the plant itself. The shrunken masses are often irregular in shape and at times angular.

Occasionally the cytoplasm is cleaved into two or several parts (fig. 10) without the formation of a cross wall. The parts may differ in color and granulation; they may be widely separated in the cell, lie in close proximity, or be superimposed.

### Comparison of foot cells with gametophytic tissues

In nearly every respect, the cells of the foot differ from those of the calyptra, involucre, and subcalyptral pad. These differences revolve around the shape, size, and arrangement of the cells; the thickness, color, composition, and continuity of the walls; the size, prominence, and number of nuclei; the granulation, color, cleavage, and plasmolysis of the cytoplasm; and finally the absence of vacuoles.

However, the cells of the gametophyte in the one to three rows adjacent to the foot may become somewhat modified (figs. 13, 14). This is particularly noticeable in the slight thickening and darkening of their walls and in the slightly increased granulation of their cytoplasm. They neither increase in size nor become multinucleate, nor are the other changes described for the cells of the foot visible in them. Nevertheless, such modifications as are present sometimes make it difficult to be certain of the exact boundary line between foot and gametophyte. Again, nearly all the morphological aspects discussed for the foot of *Riella americana* appear to be absent from the foot of almost every other genus of liverwort or moss; apparently they are absent also from the foot of other species of *Riella*.[1]

## Degeneration of foot

The time at which degeneration begins in the foot is difficult to determine. Some phases, at least, appear long before the foot reaches its mature size. This is true of the coarse granulation of the cytoplasm, of severe plasmolysis, and of the partial breakdown of certain walls. Most of the cells affected, however, continue to increase in size and reach their maturity before final decomposition sets in. Occasionally growth of the entire foot as well as of the sporange is retarded, presumably by an earlier or a more severe onset of degeneration, so that the mature foot is a dwarf with cells which are smaller than usual (figs. 11, 15). Once the foot has reached its mature size, no further changes take place in its general outline (fig. 16). The alterations accompanying degeneration are mostly protoplasmic.

Discontinuous, overlapping, and partial walls, already discussed, are a part of cell degeneration. Much of this breakdown has taken place before the foot has reached its maximum size. Nearly all the walls remain intact and rigid, and the cells retain their shape to the end.

There is little or no change in the size of the nucleus. The first noticeable modification in this structure is a cloudiness and granulation of the perinucleolar zone (cf. figs. 4 and 13). The nucleolus, which in its prime stains brilliantly, becomes muddy black in color. In later stages it and the surrounding granules disappear, leaving an apparently empty nuclear membrane; or the membrane and granules may disappear first, leaving a discolored nucleolus. Finally all traces of the nucleus vanish (fig. 16), often before the cytoplasm has completely degenerated.

Granulation of the cytoplasm continues uninterruptedly, sometimes accompanied by cytoplasmic cleavage. There may be a massing of the granules, which occurs in any part of the cell, frequently against the cell wall. The masses of granules gradually become smaller and less dense and ultimately disappear completely, leaving a lumen empty of all solid materials (fig. 16). True vacuoles may

[1] Uninucleate subdigitate, digitate, and branched cells are illustrated in the foot of *Riella affinis* by Thompson (Amer. Jour. Bot. **29**: 278. 1942).

be said to have disappeared with severe plasmolysis and clumping of the cytoplasm. Such open spaces as appear in the degenerating cytoplasm can hardly be called vacuoles. To the very end, the contact between foot and gametophyte is a very intimate one, with little or no evidence of intercellular spaces and none whatever of mucilage (fig. 16).

The final result of these degeneration phases is a foot composed of empty cell walls which, however, remain rigid (fig. 16). Degeneration is virtually complete by the time the sporange is mature and sometimes before the spores are ripe. At this time the seta may be eighteen to twenty-three cells long and a yellowish brown color.

With ripening of the sporange, it breaks off at the upper end of the seta, leaving this organ and the foot imbedded in the subcalyptral pad. Sometimes the seta, which has been yellow and apparently nonfunctional since early stages, breaks off later at its lower end. Calyptra and involucre break off at their bases. Ultimately, if the gametophytic plant continues growing (which is usually the case), seta, foot, and subcalyptral pad are shed, followed by a smoothing over of the axis of the plant at this point.

## Discussion

The anomalous structures described in this paper for the foot of *Riella americana* have been found, with only minor variations of degree, to be present in every foot examined. They may be interpreted along two major lines, in both of which the outstanding consideration is the established nutritional independence of the sporophyte of this species during the latter half of its development.

FUNCTION OF FOOT.—Present ideas of the function of the foot have been much modified from the older theory of anchorage in the gametophyte and absorption of water and elaborated food materials from that generation. Since *R. americana* is an aquatic plant, it constitutes an ideal object for the study of the nutrition of the sporophyte. In simple tap-water culture, lacking added minerals or nutrients, it has been found (**27**) that the immature as well as the mature excised sporange can absorb all the necessary water and mineral substances directly from the surrounding medium through its wall cells. It is only a step further to the absorption of these materials through an added thin calyptra, which is in direct contact with the water of the habitat. And since the sporophyte is well able to manufacture all its needed food materials, as shown in simple water culture, it seems clear that the foot, at least during the latter half of its development, does not function as an absorbing organ; or that if some absorption does take place from the gametophyte, this is purely incidental. Very little starch is present in the foot. The seta, however, gives a positive starch test before its maturity. The sporange is well supplied with chloroplasts and starch. It is probable that the developing sporange absorbs

starch from the calyptra, with which it is in intimate contact; but this also is incidental, as shown by the water cultures.

The facts that degeneration phenomena in the cells of the foot have their beginning rather early in its ontogeny, and that they are often complete before maturity of the sporophyte, lend corroborative evidence. In this species, at least, the digitate processes cannot be considered as necessarily increasing the absorbing surface, or as extending the zone from which absorption can take place, unless they represent vestigial structures from a former age during which the sporophyte was less independent from a nutritional point of view. Some other explanation would seem at least as likely.

With the elimination of the absorptive function, only the function of anchorage remains. This function it certainly performs efficiently, since the sporange is held firmly in place, even when the plant is growing on a rock in swiftly running water, which it occasionally does. Anchorage, however, is aided indirectly by both calyptra and involucre. Again, the deeply penetrating digital processes aid in anchoring, even though this also can scarcely be considered their major significance. In any event, it seems that the foot of this aquatic species now has only one major function, that of anchorage.

Possible reversal of parasitic relations.—Nutritional independence on the part of a sporophyte can be attained without the anomalous modifications of the foot described in this paper. In the genera studied by Bold (**3, 4**) the foot is apparently usually simple and unmodified, as in most of the liverworts and mosses. It seems at least possible that the significance of these modifications may lie in a reversal of the more usually accepted parasitic relations between the two generations.

There is much literature dealing with the pathological anatomy of the cells of an infected host, both plant and animal. No effort is made here to review these papers, since the general plan is well described for host plants by Küster (**17**). Affected host cells commonly undergo such modifications as enlargement to giant size; becoming multinucleate; increase in size of the nuclei; increase in mass and granulation of the cytoplasm; cleavage of the cytoplasm without formation of cross walls; thickening, resorption, and other modifications of the cell wall; and granular or other degeneration of nucleus and cytoplasm. Such conditions are summarized by Küster for wound tissue, for tissues with gummosis, and for galls induced by such varied organisms as bacteria, fungi, hemiptera, mites, and nematodes. The actual presence of the parasite is not needed to induce such changes. Watkins and Watkins (**29**), for example, show that cotton roots merely placed in contact with the mycelium of the cotton-root rot organism, or treated with extracts of infected host tissues, give similar results. It should be stressed again

that such anatomical modifications occur in the tissues of the host plant and not in those of the parasite.

The anatomical changes described in this paper for the cells of the foot of *R. americana* include phenomena very similar to those of plant host cells infected or infested with a parasite: giant, multinucleate cells; enlargement, dissolution, and other modifications of the cell walls; increase in the mass of cytoplasm; and granular decomposition of the cytoplasm and nucleus. The formation of digitate processes can well be considered a part of the formation of giant cells.

But these modified cells are in the foot, which, according to the older accepted idea, is a part of the parasitic generation. From KÜSTER's summary of the relation of host and parasite, they should be in the host generation, namely, in the gametophyte, according to the older theory. It would appear, then, that the foot in this case represents the host and not the parasite. This evidence is tentatively interpreted as indicating a reversal of the usual parasitic relationship between the generations.

It is probable, nevertheless, that the young sporophyte is for a short time partially dependent upon the gametophyte for a portion of its elaborated food supply. This is borne out by the failure of cultured sporanges to make much growth when excised during an early stage in their ontogeny (**27**). As the sporophyte grows older, however, it soon becomes completely independent, nutritionally, from the gametophyte. And the anatomical evidence here presented would seem to indicate that still later the parasitic relationship may possibly become reversed, that possibly the gametophyte exerts somewhat of a parasitic action on the sporophyte. The problem is complicated by the fact that one or two rows of cells in the subcalyptral pad lying adjacent to the foot also show a few slight modifications, especially a small amount of thickening and darkening of the cell walls. This phenomenon may at times be associated with the intimate contact between sporophyte and gametophyte and the occasional difficulty in deciding whether a given cell near the boundary line belongs to the one or the other. Often, however, cells definitely belonging to the gametophyte show a slight modification. But, in general, all the foot cells are modified as though they were affected host cells, while the cells of the surrounding gametophyte remain unmodified, or essentially so.

The hypothesis of reversed parasitism is proposed only tentatively and is based thus far only on anatomical evidence.

## Summary

1. The foot of the aquatic liverwort, *Riella americana*, arises from a broadening of the base of the young seta in a manner comparable with the early ontogeny of the foot of other liverworts.

2. A series of profound anatomical modifications begins when the foot is eight to ten cells long and proceeds uninterruptedly to its maturity and final degeneration. Most of the cells of the foot become enlarged into giant cells, which at its margin become gibbous and later commonly send digitate processes into the tissues of the surrounding gametophyte. The cell walls become thick and dark, the interior ones being sometimes partly resorbed so as to leave partial or overlapping walls. The nuclei are uniformly large; there are usually two and sometimes three or four in each cell. The cytoplasm becomes plasmolyzed and coarsely granular. Both cytoplasm and nucleus exhibit granular degeneration at or before the time of ripening of the spores.

3. The zone of contact between foot and gametophytic tissues is a very intimate one.

4. Since nutritional independence had been previously demonstrated for this species, the foot is considered as having, at least at maturity, the single major function of anchorage and none of absorption from the gametophyte.

5. Anatomical evidence, such as the formation of multinucleate giant cells and granular degeneration of the protoplasm, suggests the possibility of a reversal of the usually accepted nutritional relation between the two generations; the gametophyte of this species may prove to be parasitic upon the sporophyte, except in its early ontogeny.

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## LITERATURE CITED

1. BARTLETT, EMILY M., A comparative study of the development of the sporophyte in the Anthocerotaceae, with special reference to the genus *Anthoceros*. Ann. Bot. **42**:409–430. 1928.
2. BLAIKLEY, NELLIE M., The structure of the foot in certain mosses and in *Anthoceros laevis*. Trans. Roy. Soc. Edinburgh **57**:699–709. 1932–1933.
3. BOLD, H. C., The nutrition of the sporophyte in the Hepaticae. Amer. Jour. Bot. **25**:551–557. 1938.
4. ———, The nutrition of the sporophyte in the Musci. Amer. Jour. Bot. **27**:318–322. 1940.
5. BOWER, F. O., Primitive land plants. London. 1935.
6. BUCH, H., Morphologie und Anatomie der Hepaticae. In VERDOORN'S Manual of bryology. The Hague. 1932.
7. CAMPBELL, D. H., The development of *Geothallus tuberosus* Campbell. Ann. Bot. **10**:489–510. 1896.
8. ———, A remarkable development of the sporophyte in *Anthoceros fusiformis* Aust. Ann. Bot. **38**:473–483. 1924.
9. CAVERS, F., A new species of *Riella* (*R. capensis* from South Africa). Rev. Bryol. **30**:81–84. 1903.
10. DOUIN, CH., Le pédicelle de la capsule des Hépatiques. Bull. Soc. Bot. de France **55**:194–202; 270–277. 1908.

11. GOEBEL, K., Archegoniatenstudien: Die einfachste Form der Moose. Flora **76**:92–116. 1892.
12. ———, Archegoniatenstudien: Beiträge zur Kenntnis australischer und neuseeländischer Bryophyten. Flora **96**:1–202. 1906.
13. ———, Organographie der Pflanzen. Part 2. Jena. 1930.
14. GYÖRFFY, I., Über Endorhizoiden von *Molendoa Hornschuchiana*. Hedwigia **49**:101–103. 1910.
15. HOWE, M. A., and UNDERWOOD, L. M., The genus *Riella*, with descriptions of new species from North America and the Canary Islands. Bull. Torr. Bot. Club **30**:214–224. 1903.
16. KIENITZ-GERLOFF, F., Vergleichende Untersuchungen über die Entwickelungsgeschichte des Lebermoos-Sporogoniums. Bot. Zeitung **32**:193–203. 1874.
17. KÜSTER, E., Pathologische Pflanzenanatomie. Jena. 1925.
18. LANG, W. H., On the sporogonium of *Notothylas*. Ann. Bot. **21**:201–210. 1907.
19. LEITGEB, H., Untersuchungen über die Lebermoose. IV. Graz 1879.
20. LORCH, W., Über die Saugzellen im Fusse und in der Vaginula bei den Lebermoosen. Ber. deutsch. Bot. Ges. **43**:120–127. 1925.
21. ———, Über die Haustorialschläuche am Fusse der Laubmoose. Ber. deutsch. Bot. Ges. **43**:262–270. 1925.
22. MEYER, K., Untersuchungen über den Sporophyt der Lebermoose. I. Entwickelungsgeschichte des Sporogons der *Corsinia marchantioides*. Bull. Soc. Impér. Natural. Moscou **1911**:263–286. 1912.
23. ———, Untersuchungen über den Sporophyt der Lebermoose. II. Die Entwicklungsgeschichte des Sporogons bei *Plagiochasma*. Bull. Soc. Impér. Natural. Moscou **1913**:597–615. 1914.
24. ———, Untersuchungen über den Sporophyt der Lebermoose. III. Das Sporogonium der *Corsinia marchantioides* Raddi. Ber. deutsch. Bot. Ges. **32**:262–266. 1914.
25. O'KEEFE, LILLIAN, Structure and development of *Targionia hypophylla*. New Phytol. **14**: 105–116. 1915.
26. PORSILD, M. P., Sur une nouvelle espèce de *Riella* (subgen. nov.: *Trabutiella*) de l'Asie centrale. Bot. Tidsskr. **24**:323–327. 1902.
27. STUDHALTER, R. A., Independence of sporophyte in *Riella* and *Sphaerocarpus*. Ann. Bryol. **11**:153–154. 1938.
28. VRABER, M., Donos k poznavanju rodu *Riella*. Prirod. Razp. **2**:125–164. 1933.
29. WATKINS, G. M., and WATKINS, M. O., A study of the pathogenic action of *Phymatotrichum omnivorum*. Amer. Jour. Bot. **27**:251–262. 1940.

# MACRO-ELEMENT NUTRITION OF THE TOMATO PLANT AS CORRELATED WITH FRUITFULNESS AND OCCURRENCE OF BLOSSOM-END ROT

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(WITH ELEVEN FIGURES)

## Introduction

In the summer of 1941 an extensive experiment in plant nutrition was designed in order to examine the effects of varying concentrations of macro-nutrient elements in the nutrient medium on the ascorbic-acid content of tomato fruits. These results are reported elsewhere (**8**). Since blossom-end rot was particularly prevalent in certain treatments, it was of interest to collect and compile data on this disease. These data are reported in this paper.

Blossom-end rot of tomatoes was first reported by GALLOWAY (**7**), and the symptoms were described in some detail. Since then this disease has been recognized as occurring generally throughout the United States, Canada, Australia, New Zealand, and various parts of Europe. Some early investigators (**12, 15, 17**) attributed the pathic condition to the presence of one or more species of *Macrosporium* and *Fusarium* organisms, while SMITH (**18**) and others reported bacteria as the pathogen. Later FULTON (**6**) and BROOKS (**2**) verified the contention of SELBY (**16**) that no organisms were involved in the initial stages of the disease. More recently the occurrence of the rot has been correlated with such factors as soil moisture and water supply of the plant (**3, 5, 9, 20, 21, 23**), specific ion effects (**9, 13, 25**), and the osmotic concentration of the nutrient solution (**9, 14**). Modifying effects of nitrogen supply, carbohydrate content, length of day, light intensity, transpiration rates, bordeaux sprays, and varietal differences have also been reported. From the mass of more or less contradictory evidence which has accumulated, the viewpoint most widely accepted is that blossom-end rot is caused by a water deficit in the plant which results in a withdrawal of water from the fruit, with subsequent collapse of certain cells. As a result of this initial collapse, fungi or bacteria may be present in later stages of the disease.

## Material and methods

The strain of Bonny Best variety of tomatoes used had been inbred for seven generations.[4] Seed was planted in the greenhouse May 12, 1941, in 2-quart glazed

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[4] Seed supplied through the courtesy of Dr. LEROY POWERS, U.S. Horticultural Station, Cheyenne, Wyoming.

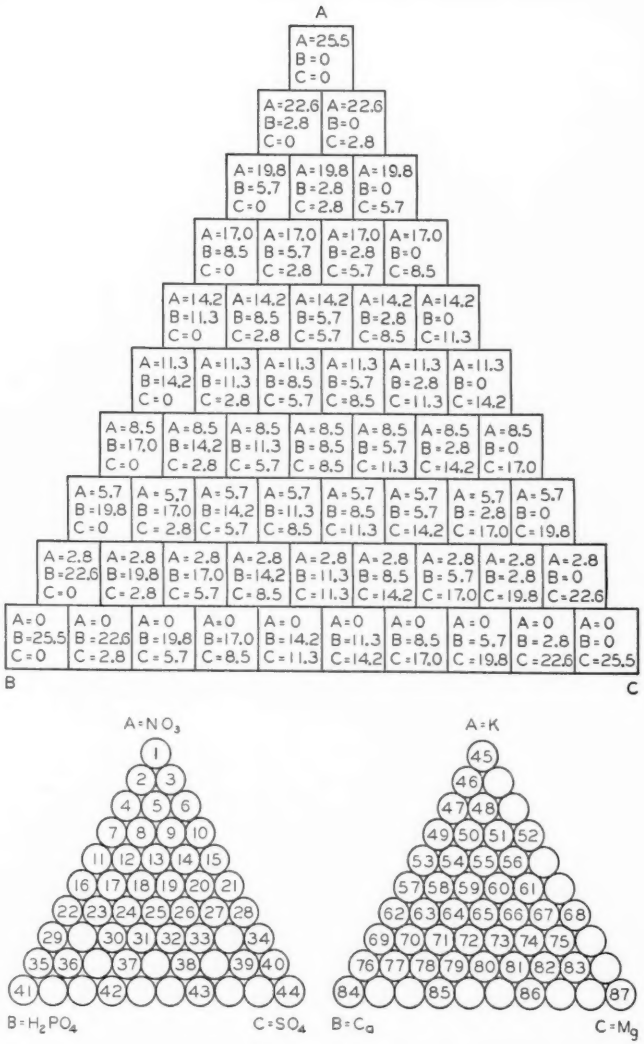


FIG. 1.—Upper triangle represents relative proportions of macro-nutrient elements in milliequivalents per liter for 55 possible treatments. Left, below: 44 combinations of varying anion concentrations. Right, below: 43 combinations of varying cation concentrations. Combinations indicated by treatment numbers.

crocks containing pure quartz sand. Two seeds were planted in each crock, and a complete nutrient solution (treatment 25, fig. 1) was used during germination and seedling stage. On June 9, when the plants were 28 days old and approximately 3 inches tall, uniform seedlings were transplanted into 2-gallon glazed crocks containing pure quartz sand, one seedling per crock. They were watered in with distilled water and immediately supplied with their respective nutrient treatments. On July 1 all plants were placed outdoors, the crocks being supported on wooden blocks. The plants were trained upright, both crocks and plants being supported by the stakes. All axillary growth was pruned off twice weekly. The plants were harvested September 10.

The design of the experiment was that of a randomized block (**4**), with eighty-seven treatments and four replications. Each replication consisted of a three-plant row, and the replications were randomized by the use of TIPPETT'S randomization tables (**22**). Thus the mean of twelve plants is used as an estimation of the results produced by a given treatment. The data were reduced by means of the analysis of variance, and the *t* test (**19**) was used for determining whether particular differences were significant. Odds as great as or greater than 19:1 were accepted as statistically significant that the observed deviations were not due to the errors of random sampling. Subsequent to the time the seedlings were transplanted, the same randomization and design was maintained, both in the greenhouse and in the field.

The method of preparing nutrient solutions has been previously described (**8**). Each group of twelve plants was supplied subsequent to the seedling stage with one of eighty-seven different nutrient solutions which varied in the relative proportions of macro-nutrient elements. Thus a total population of 1044 plants was used. All solutions contained equal amounts of the micro elements in the following concentrations:

| | |
|---|---|
| B as $HBO_3$ | 0.5 p.p.m. |
| Mn as $MnCl_2$ | 0.5 |
| Zn as $ZnSO_4$ | 0.05 |
| Cu as $CuSO_4$ | 0.02 |
| Fe as ferric citrate | 5.0 |

The relative proportions of potassium, calcium, and magnesium were varied in forty-four of these solutions, each of which contained 12.0 milli-equivalents per liter of nitrate, 4.5 m.e./l. of phosphate, and 9.0 m.e./l. of sulphate. In the remaining forty-three solutions the relative proportions of nitrate, phosphate, and sulphate were varied, while each contained 12.0 m.e./l. of calcium, 4.5 m.e./l. of potassium, and 9.0 m.e./l. of magnesium (fig. 1). For convenience, the nutrient solutions are represented in cation and anion triangles, and treatment numbers are

assigned to those solutions which were used. For purposes of discussion the treatments devoid in one or more ions will be designated as deficient.

The mean daily temperature for the duration of the experiment was 67.7° F., with a mean maximum of 80.9° and a mean minimum of 55.3°. The mean maximum temperatures in June, July, and August were 79.8°, 84.6°, and 79.3° F., respectively. The mean relative humidity from May 12 to September 10 was 45.2 per cent at 1:30 P.M. The average daily total of solar radiation for this period was 520.7 gram calories per square centimeter of horizontal surface.

## Experimentation and results

On July 25 the plants were 74 days old and had received their respective nutrient treatments for 46 days. At this time the fruits on each plant were counted and the number recorded. The fruits showing blossom-end rot were immediately harvested and the percentage of total fruit per plant computed. Subsequent to this time the entire population was examined daily and any diseased fruits discarded. Fruit started to ripen in late July and early August, and each fruit was picked on the morning of the day that complete color change occurred. When picked, a fruit was immediately weighed. Comparable portions of the first two fruits to mature on each plant were dried at 60° C. for 4 days and selected chemical analyses made. Records were kept of the weight of each fruit as it was picked in order to compile data on total fruit production and mean fruit size. On September 10, when the plants were 121 days old and had received their respective nutrient treatments for 93 days, all fruits were picked, counted, and weighed.

Since the experiment had been specifically designed so that data obtained for any character could be analyzed for the presence or absence of statistically significant differences, analyses of variance were computed for each individual character. The results are given in table 1. In characters A and B, one replication in one treatment was discarded as a result of mechanical injury, and YATES formula (24) was used for the estimation of the missing plot in order to maintain orthogonality.

It is evident (table 1) that statistically significant differences between treatments are inherent in the data. It is also evident that differences in environment when measured by replication produced significant differences in such characters as number of fruits set on July 25, mean number of fruits ripened per plant, and number and percentage of diseased fruit per plant. Since replication and randomization were essential to the design of the experiment, and since replication differences were measurable and not included in inter-treatment comparisons, valid differences exist which can be directly ascribed to treatments.

The data for the mean number of fruits set per plant on July 25, together with those for the occurrence of the rot at that time, are presented in figures 2 and 3. The greatest number of fruits set per plant in the anion triangle occurred in treat-

ments which contained 14.2–17.0 milliequivalents of nitrate per liter of nutrient medium. When lower concentrations of nitrate were used fruit set was correspondingly less, and when nitrate was omitted from the medium fruit set was negligible. When no phosphate was supplied to the plant subsequent to the seedling stage small numbers of fruits were obtained. In this experiment, however, 2.8 milliequivalents of phosphate was sufficient to result in a maximum expression of this character under conditions of comparatively high nitrate and low sulphate supply.

TABLE 1

RESULTS OF ANALYSES OF VARIANCE ON CHARACTERS FOR WHICH DATA ARE REPORTED

| CHARACTER | F VALUE | |
|---|---|---|
| | BETWEEN TREATMENTS* | BETWEEN REPLICATIONS† |
| A. Fruitfulness: | | |
| 1. Number set 7/25/41‡ | 29.21 | 24.00 |
| 2. Total number produced per plant | 60.03 | 1.21 |
| 3. Total fresh weight | 101.51 | 1.70 |
| 4. Mean number ripened per plant | 31.85 | 5.50 |
| 5. Average fresh weight of all fruits (regardless of state of maturity) | 44.62 | 1.51 |
| 6. Average fresh weight of mature fruits | 33.45 | 0.85 |
| 7. Number of days from planting to date of first fruit ripe | 11.91 | 0.79 |
| B. Occurrence of blossom-end rot: | | |
| 1. Number of diseased fruits per plant 7/25/41 | 24.94 | 8.61 |
| 2. Percentage diseased per plant 7/25/41 | 14.01 | 8.77 |
| C. Chemical analyses on first mature fruits:§ | | |
| 1. Calcium | 7.90 (1.63) | 0.88 (2.72) |
| 2. Potassium | 37.44 (2.40) | 2.36 (3.10) |
| 3. Magnesium | 9.34 (2.08) | 0.79 (2.91) |

* F value of 1.31 required for P value of 0.05: when F = 1.49, P = 0.01.
† F value of 2.64 required for P value of 0.05: when F = 3.86, P = 0.01.
‡ Subsequent to this time all fruits contracting the rot were harvested immediately.
§ For the chemical analysis, the F value required for P value of 0.05 is indicated in parentheses.

Of the plants grown in anion deficiencies, some sulphate-deficient treatments resulted in fruitful plants at this time. In treatment 22 fruitfulness is not significantly less than maximum, and concentrations of sulphate ranging from 2.8 to 11.3 m.e./l. were sufficient to result in maximum values in regard to this character.

In the anion triangle the rot, if it occurred at all, was not severe, and in most treatments there was no evidence of the disease. It is apparent that approximately 10 per cent of the fruits were diseased, in some of the treatments where maximum fruit set was observed. There was no trend which could be correlated with any anion effect.

The number of fruits set per plant at this time in treatments of the cation tri-

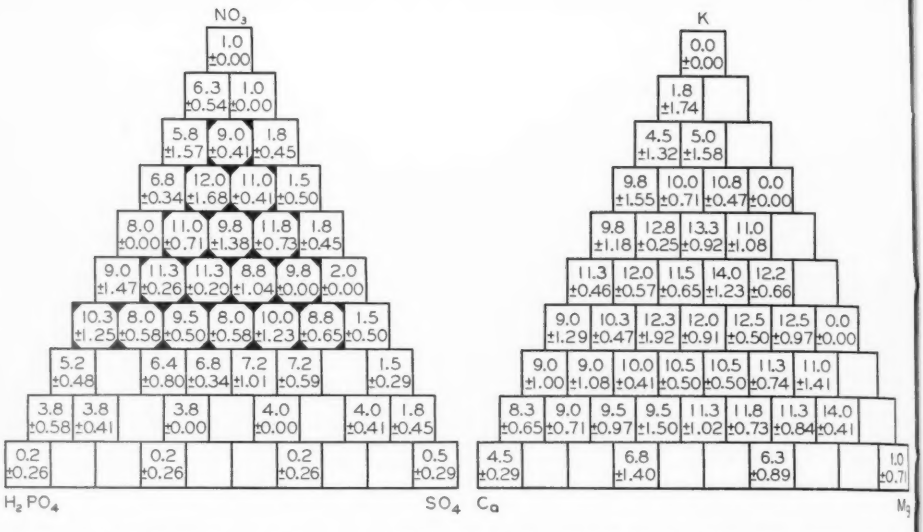


FIG. 2.*—Mean number of fruits set per plant on July 25 for anion (left) and cation (right) treatments.

* Treatment means, together with standard error. In any inter-treatment comparison, six degrees of freedom are available. When $t = 2.45$, $P = 0.05$. In this and all succeeding figures, results are given in triangular positions corresponding to equivalent positions in fig. 1.

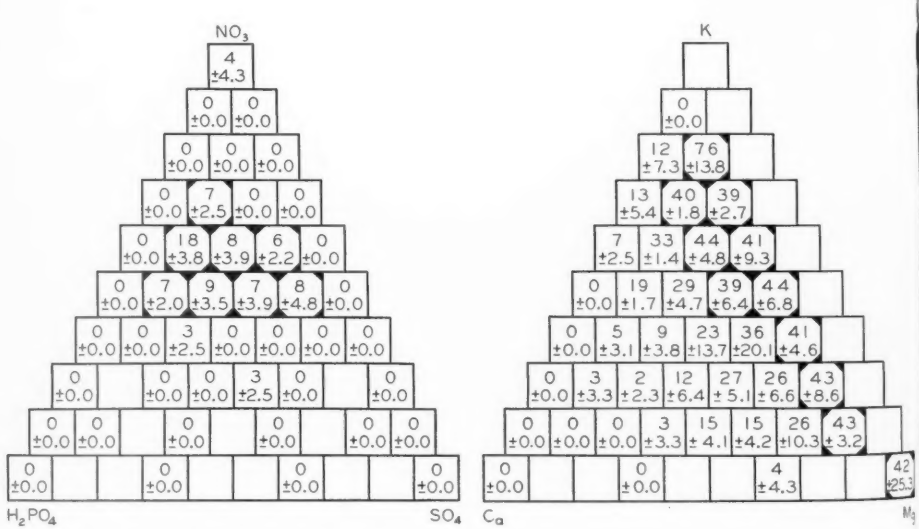


FIG. 3.—Percentage of diseased fruits per plant on July 25 for anion (left) and cation (right) treatments. See footnote to fig. 2.

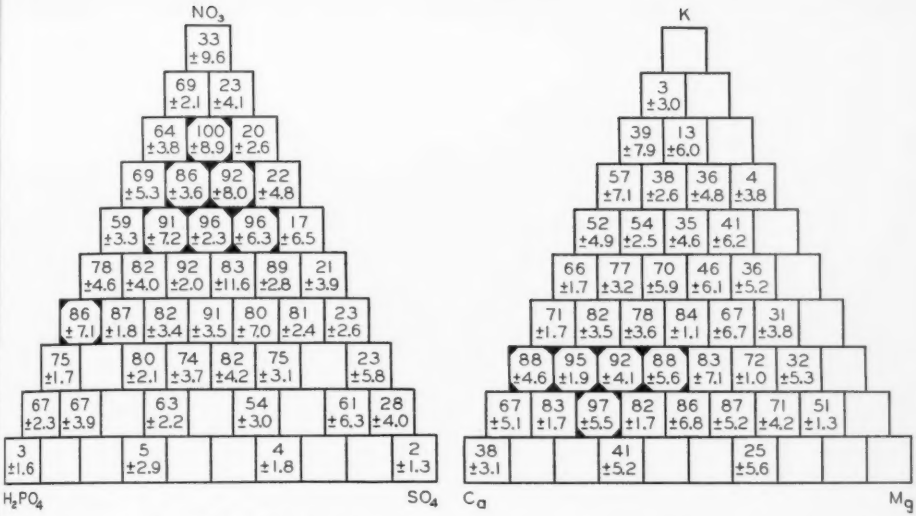


FIG. 4.—Average fresh weight of all fruit (grams) regardless of state of maturity at conclusion of experiment. Anion (left) and cation (right) treatments.

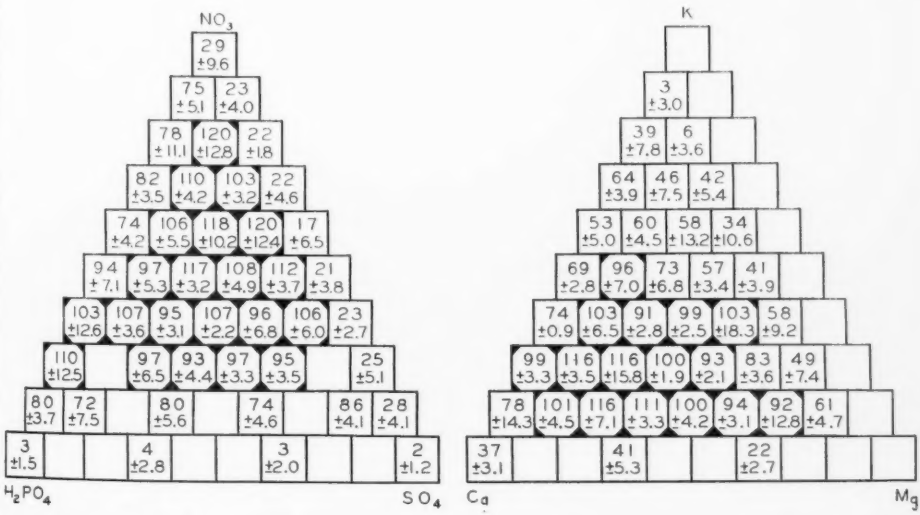


FIG. 5.—Average fresh weight of mature fruit only (grams) at conclusion of experiment. Anion (left) and cation (right) treatments.

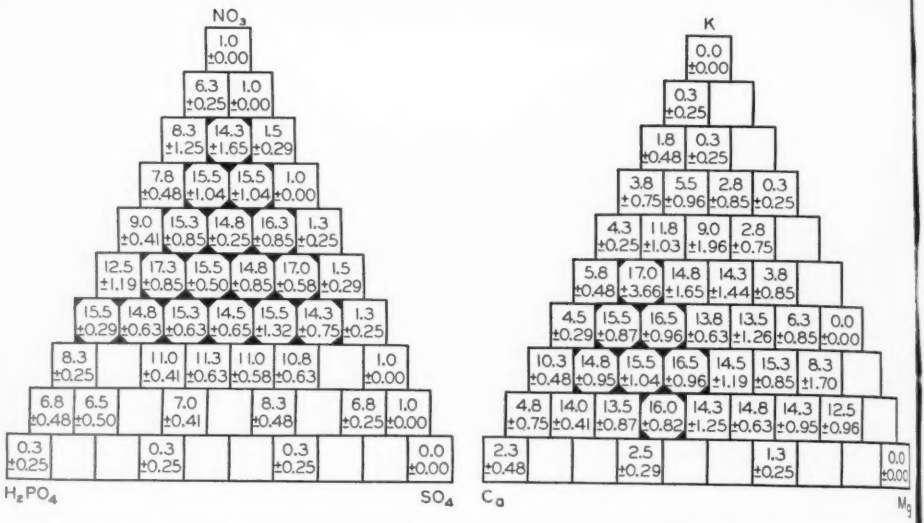


FIG. 6.—Total number of fruits regardless of state of maturity produced per plant at conclusion of experiment. Anion (left) and cation (right) treatments.

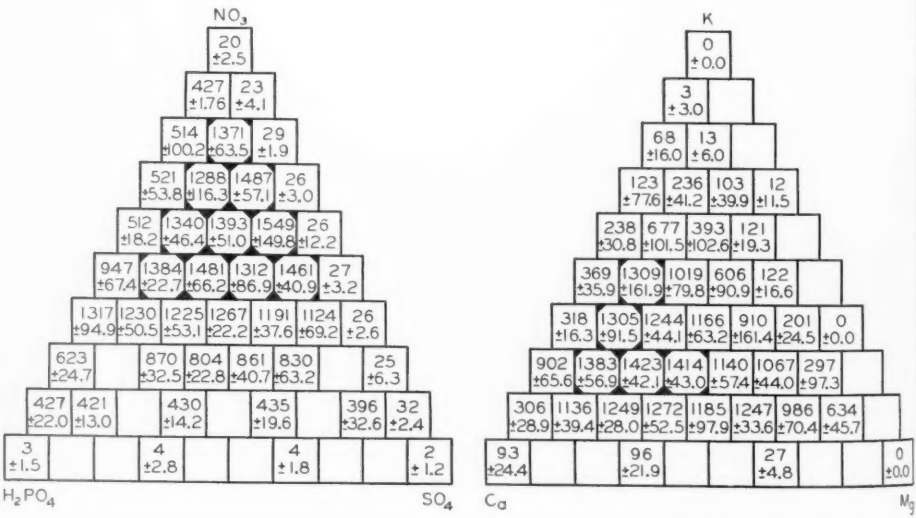


FIG. 7.—Total fresh weight of fruit per vine (grams) for anion (left) and cation (right) treatments

angle was not significantly affected by wide variations in relative composition of the nutrient medium. Significantly fewer fruits, however, were set in treatments deficient in one or more of the macro-nutrient cations. Since growing points were dead in calcium-deficient treatments, the number of fruits was less than in any other treatment. Fruit set was also significantly less than maximum in most of the potassium- and magnesium-deficient treatments, although some magnesium-deficient treatments are not affected in this respect. This observation will be discussed later.

The data for the occurrence of blossom-end rot in the cation triangle (fig. 3) showed a significant trend. As the concentration of calcium in the nutrient medium was less, the percentage of diseased fruits on each plant was correspondingly greater. In general, treatments containing 2.8 milliequivalents of calcium per liter of nutrient medium resulted in the greatest number of diseased fruit per plant and in the greatest percentage of diseased fruit per plant. There is an indication (treatments 48, 50, 55, 60) that relatively high concentrations of potassium and low concentrations of magnesium are associated with a greater percentage of diseased fruits at all calcium levels. This generalization is not applicable to magnesium-deficient treatments at comparable calcium concentrations, however, and the significance of the generalization is doubtful. If treatments deficient in one or more of the macro-nutrient cations are not considered, a significant correlation between the amount of calcium in the nutrient medium and the percentage of diseased fruit is demonstrable ($r = -0.90$, $t = 10.29$). The first diseased fruits occurred in treatments containing low concentrations of calcium. Subsequent to July 25, the greatest number of diseased fruits developed in the same treatments and accordingly were picked as the condition became apparent. In treatments containing greater amounts of calcium, the incidence of diseased fruits was correspondingly less subsequent to the initial harvest, and the diseased condition developed later than in those treatments represented by shaded areas in the triangles.

Additional data concerning fruitfulness are presented in figures 4–8. These data, which include the total weight and number of fruits produced, as well as fruit size, were compiled in an attempt to evaluate other characters of fruitfulness in so far as they could be correlated with the occurrence of the rot. The shaded portions of the triangles represent treatments where a maximum response was obtained for the character under consideration, and it is evident that, in general, the same treatments fall in the shaded portions for each character. These characters will be discussed together. The data for earliness (fig. 9) will be discussed separately.

In any interpretation of the data, it is well to note that all diseased fruits were harvested on July 25, and all fruits which set and were diseased subsequent to this time were immediately picked and discarded. Thus the following data pertain only to healthy fruits, and the data for the anion and cation triangles are discussed

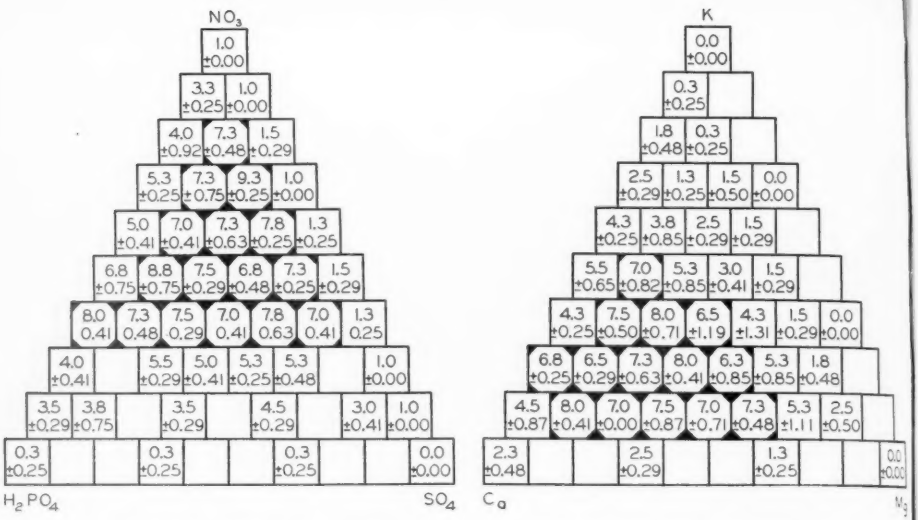


FIG. 8.—Number of fruit ripened per plant for anion (left) and cation (right) treatments

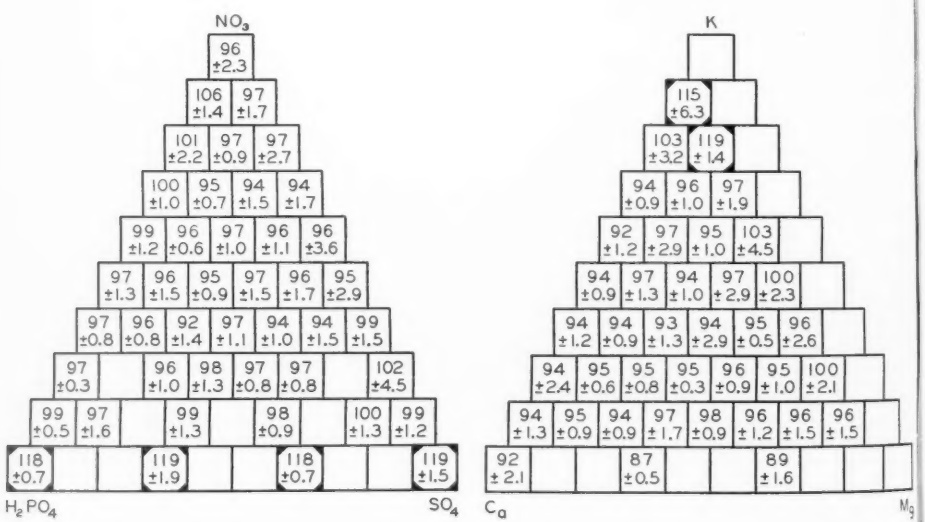


FIG. 9.—Number of days from planting to date of first fruits mature for anion (left) and cation (right) treatments

individually. In any comparison of figures 2 and 6, abscission at the pedicel joint of developing fruits is also a factor to be considered.

The trends in data for fruit characters in the anion triangle are uniform, and, in general, closely coincide with those previously discussed for the mean numbers of fruits set per plant on July 25. It should be emphasized, however, that these data were collected later in the life span of the plant, and the magnitude of the differences is increased. It is apparent that in those treatments where the rot was prevalent, maximum fruit characteristics were also observed.

In the cation triangle, however, the rot was not greatest in treatments which were the most fruitful. Relatively high concentrations of calcium (11.3–19.8 m.e./l.) are associated with maximum fruitfulness. When the concentration of calcium in the nutrient medium is less, fruitfulness is correspondingly less and the occurrence of diseased fruits correspondingly greater.

The validity of this correlation is further supported by the fact that the actual number of diseased fruits in low-calcium treatments (where fruitfulness was less) was greater than the number of diseased fruits in treatments supplied with greater amounts of calcium in the nutrient medium.

In so far as the date on which the first fruit ripened is concerned (fig. 9), wide variation in nutrient composition in the anion triangle produced no significant effect. When nitrate was absent in the media, however, the maturation of fruit was significantly slower. In the cation triangle the data for earliness showed little or no variation. There was indication, however, that a high potassium content in the nutrient medium inhibits fruit ripening and that a condition of potassium deficiency will hasten the ripening process. Fruits produced under these conditions are abnormally small.

Inasmuch as mineral composition of the nutrient medium may not be correspondingly reflected in the composition of fruits, chemical analyses are desirable to support or aid the interpretation of a correlation such as is presented here. Accordingly, analyses for the calcium content of fruits were made in certain treatments as indicated in figure 10, and the results of selected magnesium and potassium analyses are presented in figure 11.

Since diseased fruits were not available in all treatments, and since it is difficult to obtain such fruits at comparable stages of physiological development, the first healthy ripe fruit on each plant was selected for analysis. Such comparable fruits should reflect the conditions of the plant at a particular stage of physiological maturity. This condition would logically be associated with nutrient composition.

On the morning of the day that complete color change had occurred, the fruit was picked and immediately weighed. After a transverse section from the equatorial portion of the fruit was removed for vitamin analyses, the remaining apical and stem-end portions were weighed and dried at 60° C. for 96 hours. Such sam-

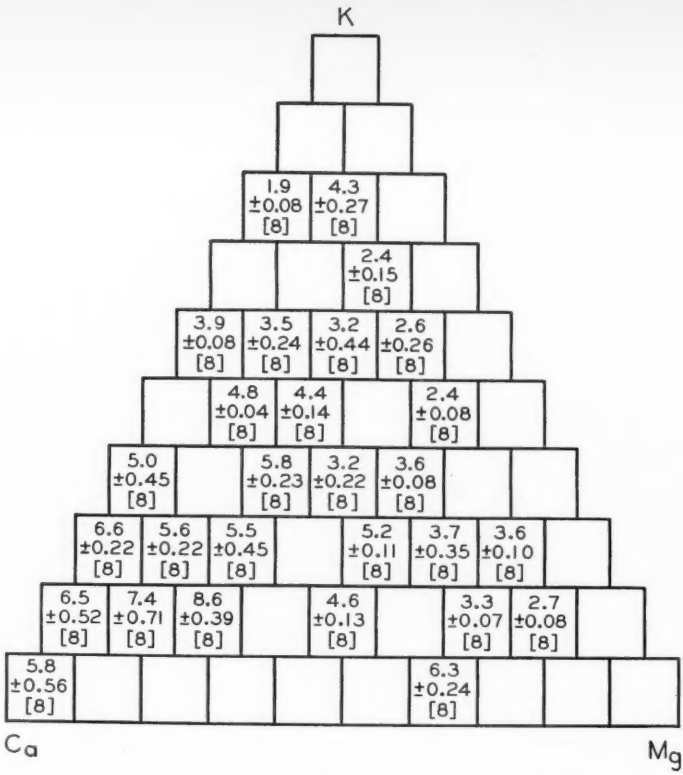


FIG. 10.—Calcium analyses on first healthy ripe fruit on each plant of treatments in cation triangle. Results in terms of milliequivalents per kilogram fresh weight of fruit. Number of analyses in each treatment indicated in brackets.

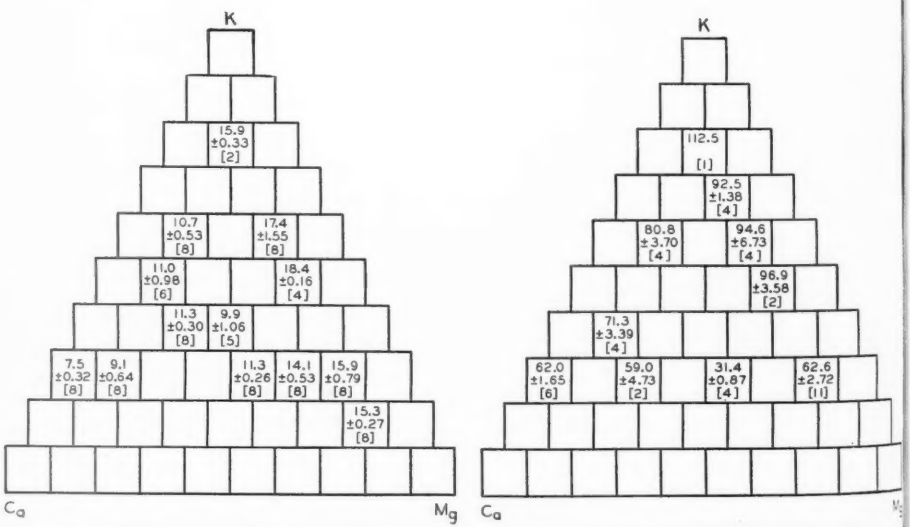


FIG. 11.—Same as fig. 10, except that analyses are for magnesium (left) and potassium (right)

ples included approximately 60–75 per cent of the fruit. The determinations of calcium, magnesium, and potassium were made according to the official methods of the A.O.A.C. (1), and the results are compiled in terms of milliequivalents of anion per kilogram fresh weight. The fruits analyzed were selected from treatments which represent wide variations in nutrient composition when all cations are considered.

From figure 10 it is apparent that, in general, a comparatively low concentration of calcium in the nutrient medium results in a low concentration of calcium in the fruit. Treatment 48 does not conform to this generalization, and it should be stressed that abnormally small fruit occurred at this point (figs. 4, 5). More than 10 per cent of the fruits were diseased in those treatments in which the first ripe fruits contained less than 5.5 milliequivalents of calcium per kilogram. Such relatively low concentrations of calcium were found in the fruit when the nutrient medium contained 2.8–11.3 milliequivalents of calcium per liter. The results of magnesium and potassium analyses (fig. 9) indicate comparatively high concentrations of both ions in fruits from treatments where the incidence of the disease was high. Although lower concentrations of both ions occurred in fruits from other treatments, a direct relationship with the occurrence of the rot was not apparent.

The ratios Mg:Ca and K:Ca were particularly high in the low calcium treatments (51, 56, 61, 75, and 83). This would be expected, when the nutrient composition is considered, and the significance of these ratios to diseased fruit is not therefore evident.

A considerable amount of magnesium is present in fruits of treatment 69. The plants of this treatment were supplied with no magnesium subsequent to the seedling stage. This condition may represent an accumulation in the first fruit of a portion of the magnesium which was supplied early in the life span of the plant. In fruits which subsequently matured, less magnesium would be expected. This hypothesis is being tested by analyses of the vegetative portions of the plant and of fruits which subsequently developed. A greater number of treatments, involving all the macro-nutrient elements, are under consideration in an attempt to clarify the influence of the relative composition of the nutrient medium on ion absorption. The results will be presented in a later paper.

## Discussion

When the characters listed in table 1 are used as criteria of fruitfulness, it is apparent that maximum responses are obtained in a definite group of treatments and that optimum nutrient composition in this experiment can be designated within narrow limits. In general, variations in calcium and nitrate produced more apparent differences over wider ranges in concentrations than were obvious with other macro-nutrient elements. Greatest fruitfulness occurred when the concen-

tration of calcium in the nutrient medium was comparatively high. Similarly, as the concentration of nitrates was increased from 0.0 to 8.5 m.e./l., fruitfulness was correspondingly greater. It is recognized, however, that nutrient composition which is optimum for fruitfulness may not be optimum for such characters as height of plant and fresh and dry weight of root system.

The interaction of certain ions is obvious. Thus, at concentrations of calcium of 5.7 milliequivalents, greater fruitfulness resulted when potassium was relatively low and magnesium relatively high. Other examples could be cited, and the interactions are particularly apparent in treatments completely lacking one or more ions.

The occurrence of blossom-end rot is clearly correlated with calcium nutrition. In the cation triangle, with decreasing concentrations of calcium in the nutrient medium the percentage of diseased fruits was correspondingly greater. The calcium content of fruits was associated with the amount of calcium supplied to the plant. Fruits which contained the smallest amounts of calcium were produced in treatments where the disease was most prevalent. No correlation of the occurrence of this rot with any other macro-nutrient element was observed. The percentage of diseased fruits in certain treatments of the anion triangle closely approximated values for treatments of similar nutrient composition in the cation triangle. Thus treatment 19 in the anion triangle, which closely corresponds to treatment 72 in the cation triangle in so far as nutrient composition is concerned, shows the same percentage of diseased fruits within the limits of error ($7 \pm 3.9$ and $12 \pm 6.4$, respectively).

The results reported here are in agreement with the observations of RALEIGH (13), using aerated nutrient culture solution with and without calcium. YOUNG (25) observed relatively less blossom-end rot with the application of potassium fertilizers to soils. The data reported here show no primary effect of potassium in sand culture which would support this viewpoint. HOFFMAN (10) reports that the application of potash fertilizers to soils increases the occurrence of the rot only to a limited degree. This is in agreement with the present data.

It is recognized that there is no simple explanation for the occurrence of this disease. However, two related factors, such as calcium nutrition and moisture relations of the plant, might be used as a basis for the correlation of other observations.

Whether one of these factors functions independently of the other is a question not answered in the literature. In this experiment it might be reasoned that root development in pot culture is possibly not comparable with soil conditions. Such restricted root development might result in less water absorption by the plant. If this were the only factor involved, it seems probable that all variations in nutrient supply would result in the same percentage of diseased fruits. It might

further be reasoned that when a relatively small amount of calcium was supplied in the nutrient medium, less extensive root growth—and hence less water absorption—occurred. That this hypothesis is untenable is shown by the fact that both vine and root growth, as measured by fresh- and dry-weight production, are greatest in low calcium treatments (2.8 milliequivalents of calcium per liter of nutrient medium). Furthermore, the percentage dry matter of the vine is significantly less in low calcium treatments as compared with high. These data would thus indicate that those treatments where the rot was most prevalent resulted in the most succulent vines and the most extensive root systems. Thus, in this experiment water intake was actually relatively greater in low calcium treatments.

Since no direct effect of potassium was observed in sand culture where exchange phenomena are absent, it would seem possible that a secondary effect is being observed in cases where potash applications to soils reduce the occurrence of the disease. If one were dealing with a very acid soil with a relatively small quantity of calcium in the colloidal complex, it is conceivable that an increase in the concentration of potassium ions in the soil solution would liberate some calcium in the exchange complex, making it more readily available to the plants.

KERTESZ *et al.* (11) have reported that the addition of 170 p.p.m. of calcium chloride to peeled tomatoes for a short time during the canning process will result in firmer fruits with no subsequent disintegration of fleshy portions. Their data show that an appreciable amount of calcium has been absorbed by the cells and is not subsequently lost by drainage. The drained weight is materially increased over that of untreated tomatoes. Their data seem to indicate a greater retaining capacity of cells for water or soluble materials.

The role that calcium has in water relationships within the plant is not at all clear, and the following questions might be proposed: (*a*) Does calcium in the cell walls or in semipermeable membranes influence the water-retaining capacity of cells? If so, an adequate accumulation in the fruit is necessary to prevent water withdrawal. Also, a lack of calcium in the fruit might result in a "flooding" of intercellular spaces, thereby limiting gaseous exchange for metabolism. (*b*) Is water transfer controlled by or related to the content of calcium in the cell walls? If so, do cells "compete" for water supply? The following questions might also be considered concerning calcium in the external environment: (*a*) Does an "imbalance" of the nutrient media resulting from variations in calcium supply result in differential ionic absorption? This may be indicated in the potassium and magnesium analyses of fruits in this experiment. (*b*) Is the volume of nutrient supply a factor in the absorption of water and nutrient elements?

While these questions are in no way clarified by the data presented here, their importance to an understanding of the disease is suggested.

## Summary

1. A population of 1044 plants of an inbred strain of Bonny Best tomatoes was grown in sand culture. The effects of eighty-seven different nutrient solutions varying in the relative proportions of macro-nutrient elements were studied in relation to fruitfulness and the occurrence of blossom-end rot. The data were reduced and analyzed by statistical methods.

2. In general, variations in amounts of calcium and nitrate in the nutrient medium resulted in greater differences in fruitfulness over wider ranges in concentrations than did the other elements.

3. Greatest fruitfulness occurred in treatments relatively high in nitrate and low in sulphate and phosphate in the anion triangle and in treatments relatively high in calcium and low in magnesium and potassium in cation triangle.

4. The percentage of diseased fruits on each plant increased with decreasing calcium concentrations in the nutrient medium. This correlation is largely independent of magnesium and potassium concentrations, and no correlation with any anion was observed.

5. Fruits produced in treatments where the rot was most severe were low in calcium content and high in potassium and magnesium content.

6. The occurrence of the rot was definitely associated with calcium nutrition.


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## LITERATURE CITED


1. ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.—Methods of analysis. 5th ed. 1940.
2. BROOKS, C., Blossom-end rot of tomatoes. Phytopath. **4**:345–373. 1914.
3. CHAMBERLAIN, E. E., Blossom-end rot of tomatoes. Jour. Agr. New Zealand **46**:293–296. 1933.
4. FISHER, R. A., The design of experiments. London. 1937.
5. FOSTER, A. C., Effect of environment on the metabolism of the tomato plant as related to development of blossm-end rot of the fruit. Phytopath. **29**:7. 1939.
6. FULTON, H. R., WRIGHT, W. J., and GREEG, J. W., The control of insects and diseases affecting horticultural crops. Pennsylvania Agr. Exp. Sta. Bull. 110. 39. 1911.
7. GALLOWAY, B T., Notes on the black-rot of the tomato. U.S. Dept. Agr. Rept. 1888. 339–346. 1888.
8. HAMNER, K. C., LYON, C. B., and HAMNER, C. L., Factors influencing the ascorbic-acid content of the tomato with special reference to mineral nutrition. BOT. GAZ. **103**: 586–616. 1942.
9. HAYWARD, H. E., Unpublished data; private communication.
10. HOFFMAN, J. C., The relation between water deficiency and blossom-end rot of tomatoes. Abstracts of doctor's dissertations, no. 24. Ohio State University Press. 1937.
11. KERTESZ, Z. I., TOLMAN, T. G., LACONTI, J. D., and RUYLE, E. H., The use of calcium in the commercial canning of whole tomatoes. N.Y. (Geneva) Agr. Exp. Sta. Tech. Bull. 252. 1940.

12. QUINN, G., The production of early tomatoes. South Australia Dept. Agr. Bull. **6**:15. 1905.
13. RALEIGH, G. J., Fruit abnormalities of tomatoes grown in various culture solutions. Proc. Amer. Soc. Hort. Sci. **37**:895–900. 1939.
14. ROBBINS, W. R., Relation of nutrient salt concentration to growth of the tomato and to the incidence of blossom-end rot of the fruit. Plant Physiol. **12**:21–50. 1937.
15. ROLFS, P. H., The tomato and some of its diseases. Florida Agr. Exp. Sta. Bull. 21. 36. 1893.
16. SELBY, A. D., Investigations of plant diseases in forcing house and garden. Ohio Agr. Exp. Sta. Bull. 73. 241–242. 1897.
17. SMITH, E. H., Blossom-end rot of tomatoes. Massachusetts Agr. Exp. Sta. Tech. Bull. 3. 1907.
18. SMITH, R. E., Report of the Plant Pathologist to July 1, 1906. California Agr. Exp. Sta. Bull. 184. 244. 1907.
19. SNEDECOR, G. W., Statistical methods. Collegiate Press, Ames, Iowa. 1938.
20. STOUT, G. J., Influence of watering treatment on the occurrence of blossom-end rot in greenhouse tomatoes. Proc. Amer. Soc. Hort. Sci. **32**:515–518. 1934.
21. STUCKEY, H. P., and TEMPLE, J. C., Tomatoes. II. Blossom-end rot. Georgia Agr. Exp. Sta. Bull. 96. 69–91. 1911.
22. TIPPETT, L. H. C., Tracts for computers. Random sampling numbers. Cambridge Univ. Press. London. 1927.
23. WEDGWORTH, H. H., NEAL, D. C., and WALLACE, J. M., Wall and blossom-end rot of the tomato. Mississippi Agr. Exp. Sta. Bull. 247. 1927.
24. YATES, F., The empire journal of experimental agriculture. **1**:129. 1933.
25. YOUNG, P. A., HARRISON, A. L., and ALTSTATT, G. E., Common diseases of tomatoes. Texas Agr. Exp. Sta. Circ. **86**:1–32. 1940.

# RELATIONSHIP OF DISSOCIATION OF CELLULAR PROTEINS BY AUXINS TO GROWTH[1]

HENRY T. NORTHEN

(WITH ONE FIGURE)

Protoplasm is a system which, like *in vitro* protein ones, may be reversibly dissociated by various agents (**52**). The data presented in this paper indicate that auxins condition a dissociation of cellular proteins as evidenced by decreases in the structural viscosity of protoplasm. Such dissociation, with a concomitant decrease in the molecular weight of cellular proteins and an increase in the number of active sulfhydryl groups, may condition accelerated rates of imbibition, diastatic activity, and respiration, as well as modify development.

## Material and methods

Stems and petioles from navy bean plants 8–15 days old, which had been grown in window boxes of the laboratory, were smeared unilaterally with hydrated lanolin preparations of indole-3-acetic acid, indole-3n-propionic acid, and α-naphthalene acetic acid. After the desired periods (tables 1–7), the responses were noted. Then a 1.5-cm. piece from each stem or petiole was centrifuged between wet cotton with an acceleration of 680 × gravity for 4 minutes, an acceleration which would not displace chloroplasts or starch grains in most cells of control petioles or stems. In the centrifuge tubes the axes of the plant parts were at right angles to the direction of the acceleration. Following centrifugation, freehand cross-sections were prepared and mounted in IKI. Next the percentages of cells in which the chloroplasts or starch grains were displaced on the auxin-treated sides and on the control sides were determined. In other experiments the tops of decapitated plants were daubed with lanolin or with lanolin containing growth substance. Such stems were then centrifuged, sectioned, mounted, and observed. About 4000 plants were treated, of which percentages of displacement were determined for about 500 plant parts. Approximately 150,000 cells were counted.

## Data

### Petioles treated on one side

Mixture of 1 mg. indole-3-acetic acid in 1 gm. lanolin.—In table 1, data for a typical experiment, one of three, are recorded. It will be noted that with all

[1] Contributions from the Department of Botany and the Rocky Mountain Herbarium of the University of Wyoming, no. 189.

TABLE 1

| Hours exposed | Response | Percentage of cells showing displacement in | | | |
|---|---|---|---|---|---|
| | | Cortex | | Ray | |
| | | I.a. side | Control side | I.a. side | Control side |
| 0.0 | ..... | ..... | 3 | ..... | 0 |
| 0.0 | ..... | ..... | 2 | ..... | 0 |
| 0.5 | 0* | 65 | 15 | 62 | 28 |
| 0.5 | 0 | 52 | 14 | 36 | 9 |
| 1.0 | − | 68 | 16 | 65 | 5 |
| 1.0 | − | 70 | 9 | 68 | 9 |
| 1.5 | − | 89 | 30 | 92 | 37 |
| 1.5 | − | 89 | 20 | 94 | 39 |
| 2.0 | − | 92 | 21 | 84 | 16 |
| 2.0 | − | 87 | 30 | 79 | 23 |
| 3.0 | − | 90 | 28 | 99 | 45 |
| 20.0 | − | 66 | 18 | ..... | ..... |
| 20.0 | − | 71 | 16 | ..... | ..... |

*In this and following tables: 0, none; −, growth greater on i.a. side; +, growth greater on control side.

TABLE 2

| Concentration (mg. per gm.) | Hours exposed | Response | Percentage of cortex cells showing displacement of chloroplasts on | |
|---|---|---|---|---|
| | | | I.p. side | Control side |
| 20 | 20.0 | 0 | 12 | 7 |
| | 20.0 | 0 | 26 | 18 |
| | 22.0 | 0 | 2 | 2 |
| 40 | 3.0 | − | 67 | 14 |
| | 3.5 | − | 64 | 11 |
| | 4.5 | − | 54 | 16 |
| | 5.5 | − | 80 | 10 |
| | 6.5 | − | 86 | 34 |
| 60 | 2.0 | − | 75 | 8 |
| | 5.0 | − | 81 | 9 |
| | 6.0 | − | 90 | 27 |
| | 6.5 | − | 84 | 15 |
| | 28.0 | − | 96 | 17 |
| 100 | 0.2 | 0 | 5 | 3 |
| | 0.5 | − | 71 | 7 |
| | 1.0 | − | 80 | 27 |
| | 1.5 | − | 75 | 11 |
| | 3.0 | − | 51 | 15 |
| | 5.0 | + | 6 | 85 |

exposures the chloroplasts in the cortical cells and the starch grains in the ray cells were displaced by the centrifugation in greater percentages of cells on the indole-3-acetic acid (i.a.) side than on the control side. This indicates that the structural viscosity of protoplasm has been decreased on the i.a. side. Presumably decreases in structural viscosity are conditioned by a dissociation of cellular proteins.

INDOLE-3N-PROPIONIC ACID.—In all instances (table 2) the structural viscosity was lower on the convex than on the concave side in the experiments with this acid (i.p.).

MIXTURE OF 1 MG. α-NAPHTHALENE ACETIC ACID IN 1 GM. LANOLIN.—The data from one of three experiments which agreed closely are recorded in table 3. Dur-

TABLE 3

| HOURS EXPOSED | RESPONSE | PERCENTAGE OF CORTEX CELLS SHOWING DISPLACEMENT OF CHLOROPLASTS ON | | HOURS EXPOSED | RESPONSE | PERCENTAGE OF CORTEX CELLS SHOWING DISPLACEMENT OF CHLOROPLASTS ON | |
|---|---|---|---|---|---|---|---|
| | | N.A. SIDE | CONTROL SIDE | | | N.A. SIDE | CONTROL SIDE |
| 0.0 | ..... | ..... | 5 | 3.0 | − | 55 | 10 |
| 0.0 | ..... | ..... | 11 | 3.0 | + | 82 | 81 |
| 0.5 | − | 25 | 4 | 3.0 | + | 82 | 80 |
| 0.5 | − | 54 | 21 | 4.5 | − | 93 | 47 |
| 1.0 | − | 48 | 7 | 4.5 | − | 89 | 26 |
| 1.0 | − | 65 | 24 | 4.5 | + | 67 | 72 |
| 1.5 | − | 61 | 12 | 4.5 | + | 69 | 53 |
| 1.5 | − | 78 | 7 | 24.0 | − | 57 | 30 |
| 3.0 | − | 91 | 30 | 24.0 | − | 62 | 14 |

ing the initial negative curvature the viscosity was lower on the faster than on the slower growing side. Three hours and 4½ hours after application of the n.a., some petioles exhibited a negative bending and others a positive. In the former instance the viscosity was lower on the auxin side. When the curvatures were positive, however, the viscosity was low on both sides. At the end of 24 hours all the petioles exhibited a negative bending and the viscosity was lower on the auxin side.

### PETIOLES TREATED ON UPPER OR LOWER SURFACE WITH 1 MG. OF α-NAPHTHALENE ACETIC ACID PER GM. OF LANOLIN

In this experiment percentages of displacement were determined in various areas of the cortex. In table 4 the regions are indicated by numbers. The diagram

(fig. 1) reveals the area of the petiole corresponding to each number. Regions three and six are those of collenchyma, whereas the others are of chlorenchyma. Each percentage is the average of fifteen sections cut from five petioles.

TABLE 4

| Hours exposed | Response | Surface treated | Percentage displacement in region | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 |
| 0.5 | − | Upper | 54 | 41 | 0 | 2 | 14 | 0 |
| 0.5 | − | Lower | 3 | 7 | 8 | 15 | 51 | 88 |
| 1.0 | − | Upper | 70 | 78 | 55 | 32 | 16 | 34 |
| 1.0 | − | Lower | 8 | 18 | 75 | 70 | 65 | 75 |
| 1.25 | − | Upper | 64 | 70 | 78 | 8 | 11 | 0 |
| 1.25 | − | Lower | 0 | 4 | 43 | 53 | 91 | 96 |
| 2.0 | − | Upper | 31 | 45 | 45 | 37 | 41 | 43 |
| 2.0 | − | Lower | 40 | 38 | 68 | 78 | 89 | 94 |
| 3.5 | + | Upper | 58 | 78 | 90 | 73 | 68 | 92 |
| 3.5 | + | Lower | 84 | 89 | 60 | 82 | 70 | 48 |

Initially the viscosity was decreased only in regions near the point of application. With longer exposures the viscosity was decreased in more distant regions, until at the end of $3\frac{1}{2}$ hours it was about the same in remote locations as in close ones. With the exception of the experiment where the upper surface was treated and the exposure was 2 hours, increased growth was paralleled by lowered viscosity when the curvatures were negative. When the bendings were positive, however, the differences between the two sides were not significant.

FIG. 1

## AUXINS APPLIED UNILATERALLY TO FIRST INTERNODE OF STEMS

MIXTURE OF 1 MG. INDOLE-3-ACETIC ACID IN 1 GM. OF LANOLIN.—The protoplasmic viscosity was decreased in ray cells on the i.a. side before any noticeable bending occurred (table 5). When the curvature was negative the viscosity was lower on the auxin than on the control side. After 2 hours there was little difference in the protoplasmic viscosity of the two sides.

INDOLE-3N-PROPIONIC ACID.—In general, the data (table 6) indicate that when curvatures are negative the structural viscosity of the protoplasm is lower on the convex side.

### AUXINS APPLIED ON TOPS OF FIRST INTERNODES OF STEMS

MIXTURE OF 10 MG. INDOLE-3N-PROPIONIC ACID IN 1 GM. OF LANOLIN.—In this experiment plants were decapitated just below the first pair of leaves. The cut surfaces of some of the plants were then daubed with lanolin and those of others with a mixture of lanolin and i.p. acid. Following centrifugation, sections were

TABLE 5

| HOURS EXPOSED | RESPONSE | PERCENTAGE OF RAY CELLS SHOWING STARCH GRAINS DISPLACED | |
|---|---|---|---|
| | | I.A. SIDE | CONTROL SIDE |
| 0.0 | ..... | ..... | 3 |
| 0.0 | ..... | ..... | 5 |
| 0.5 | 0 | 60 | 17 |
| 0.5 | 0 | 54 | 3 |
| 1.0 | − | 62 | 9 |
| 1.0 | − | 81 | 24 |
| 2.0 | + | 92 | 94 |
| 2.0 | + | 83 | 79 |

TABLE 6

| CONCENTRATION (MG. PER GM.) | HOURS EXPOSED | RESPONSE | PERCENTAGE DISPLACEMENT IN | | | |
|---|---|---|---|---|---|---|
| | | | CORTEX CELLS | | RAY CELLS | |
| | | | I.P. SIDE | CONTROL SIDE | I.P. SIDE | CONTROL SIDE |
| 20 | 0.0 | ..... | ..... | 3 | ..... | 5 |
| | 5.0 | − | 86 | 32 | 84 | 17 |
| 60 | 0.0 | ..... | ..... | 0 | ..... | 0 |
| | 1.0 | − | 10 | 10 | 33 | 11 |
| | 1.5 | − | 80 | 10 | 42 | 21 |
| | 24.0 | − | 72 | 10 | 96 | 38 |

cut at different distances from the surface. At the end of 22 hours slight swellings were noted at the tops of those plants which received this acid. Such protuberances subsequently increased in size. Their behavior was very much like that described by KRAUS, BROWN, and HAMNER (**36**). The experiment involving exposures of 11 and 12 hours was performed when the internodes were 3 cm. long, whereas the other experiment, involving 22- and 28-hour exposures, was per-

formed when the internodes were 4 cm. long. The data (table 7) indicate that i.p. acid conditions a decrease in structural viscosity.

MIXTURE OF 1 MG. α-NAPHTHALENE ACETIC ACID IN 1 GM. OF LANOLIN.—After an exposure of 22 hours the starch grains were displaced by centrifugation in 76 per cent of the experimental ray cells (5 mm. from the apex) and in 11 per cent of the control ray cells.

TABLE 7

| Hours exposed | Millimeters from apex | Percentage of displacement in | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cortex cells | | Ray cells | | Pith cells | |
| | | Lanolin +I.P. | Lanolin only | Lanolin +I.P. | Lanolin only | Lanolin +I.P. | Lanolin only |
| 11 | 3 | 70 | ..... | 89 | ..... | 100 | ..... |
| | 7 | 92 | 14 | 93 | 28 | 100 | 30 |
| | 10 | 90 | ..... | 100 | ..... | 100 | ..... |
| 12 | 3 | ..... | ..... | 85 | ..... | 92 | ..... |
| | 7 | 83 | 9 | 100 | 59 | 100 | 44 |
| | 10 | 86 | ..... | 91 | ..... | 97 | ..... |
| 22 | 3 | 70 | 0 | 55 | 0 | 86 | 0 |
| | 7 | 100 | 0 | 66 | 0 | 100 | 0 |
| 28 | 3 | 56 | ..... | 79 | ..... | 100 | ..... |
| | 7 | 55 | 19 | 80 | 22 | 100 | 9 |
| | 10 | 70 | ..... | 90 | ..... | 100 | ..... |

INDOLE-3N-PROPIONIC ACID APPLIED ON TOPS OF SECOND INTERNODES OF STEMS

The tops of some second internodes were treated with lanolin and others with lanolin containing 10 mg. of i.p. acid per gram. Following centrifugation, sections were cut 4 mm. from the apex. Displacements of starch grains were noted in 85 per cent of the ray cells from plants which received this acid and in 1 per cent from control plants after 5 hours' exposure, in 76 and 0 per cent, respectively, after 10 hours and in 80 and 0 per cent, respectively, after 24 hours, thus manifesting that the structural viscosity had been decreased by the acid.

## Discussion

The application of growth substances to plants may condition increased plasticity of cell walls (**26**), intussusception of new wall material (**68**), augmented swelling pressure (**72**), quickened rates of respiration (**1, 8, 58, 75, 81**) and carbohydrate hydrolysis (**5, 48**), initiation of cell division (**7, 28, 47, 67, 79**), and at times mitotic abnormalities (**20, 36**).

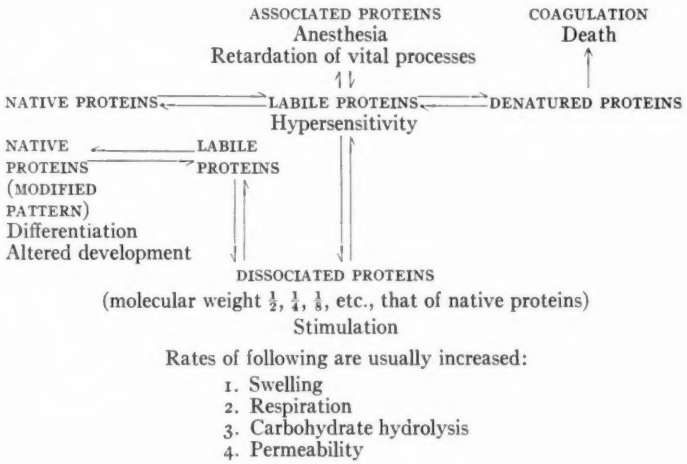
With the possible exception of wall plasticity, all the listed processes are under protoplasmic control. The rates of such processes are often increased by agents,

including growth substances, which condition a dissociation of cellular proteins. For example, carbon dioxide, anesthetics, disease, alternating currents, direct currents, warm baths, wounding, X-irradiation, soap, and sodium taurocholate initially cause a dissociation of cellular proteins, as evidenced by decreases in viscosity (3, 13, 25, 49, 50, 51, 53, 54).

Of these the following may initially increase the rate of respiration: carbon dioxide (76, 77), anesthetics (21, 34), disease (59), mechanical agents (4, 19, 31), sodium taurocholate (10), and X-irradiation (66). The hydrolysis of starch may be accelerated by carbon dioxide (77), anesthetics (33), alternating currents (40), warm baths (33), wounding (31), and X-irradiation (66). Some agents which initiate cell division in unfertilized animal eggs are carbon dioxide (27), ether (45), electrical currents (14), warm baths (44), and soaps (42). Agents which dissociate cellular proteins and produce mitotic abnormalities are anesthetics, high temperatures, wounding, disease, and X-irradiation (65).

The data presented indicate that growth substances condition a dissociation of cellular proteins. In this particular they act like many agents. It has been suggested that such dissociation conditions accelerated rates of many processes which are controlled by protoplasm.

The following diagram illustrates some of the transmutations which may occur when cellular proteins are subjected to environmental changes. The physiological resultants of each state are also listed.




Rates of following are usually increased:

1. Swelling
2. Respiration
3. Carbohydrate hydrolysis
4. Permeability

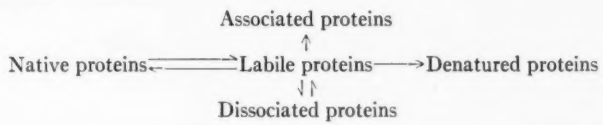
It must be emphasized that the series of reactions is not entirely hypothetical but is based on experimental data. NORTHEN (51) obtained evidence which suggested that labile proteins were formed prior to dissociation. He noted that solu-

tions of ether, so dilute that neither dissociations nor associations resulted, rendered the protoplasmic proteins more sensitive to dissociation by a mechanical impact. Apparently the dilute ether changed the native proteins into the more readily dissociable labile proteins.

Previously data have been cited which indicate that many agents decrease the structural viscosity of protoplasm. It is probable that decreases in structural viscosity are conditioned, in part at least, by dissociations of cellular proteins. First, because agents which decrease the structural viscosity of protoplasm dissociate *in vitro* proteins. The following agents which decrease protoplasmic viscosity may dissociate proteins: temperature of 35° C. (**69**), sound (**11**), amides (**22, 71**), alcohols (**70**), and X-irradiation (**6**). Second, because viscosity may be proportional to molecular weight (STAUDINGER, in SCHMIDT, **64**).

When cells are treated with some agents an increase in viscosity follows an initial decrease (**3, 25, 49, 52, 53**). It is likely that the increase is conditioned in part by an association of cellular proteins.

The concept of protoplasm as a reversibly dissociable-associable system is in harmony with biochemical evidence. The preceding diagram was modeled from that suggested by LUNDGREN and WILLIAMS (**43**) for *in vitro* proteins. They outlined the series as follows:




Prior to them, SØRENSEN (**70**) and SVEDBERG (**73**) concluded that proteins were reversibly dissociable-associable component systems. SVEDBERG states: "The lability [of proteins] reveals itself in a number of well defined and reversible dissociation-association reactions, the causes of which are changes in the environment." In other words, even *in vitro* protein systems are irritable. They can respond to relatively small environmental changes.

According to the dissociation-association idea of protoplasm, the dissociation of cellular proteins, by auxins or other agents, conditions an increased swelling pressure, which is probably a factor in the enlargement of cells. Biochemical evidence supports this deduction. NORTHROP (**55**) noted that a gel which ceased to take up water again did so if the network lost its elasticity. LLOYD (**41**), from studies on the relationship between protein structure and water absorption, concluded that the more compact the structure the less the swelling and the looser the structure the greater the swelling. KÚTHY (**38**) observed that substances which tended to change gels into sols promoted swelling, whereas those which conditioned the reverse reaction inhibited swelling. Moreover, from a thermodynamical point

of view, and in accordance with the data presented, it is highly probable that increased swelling is conditioned by a dissociation of cellular proteins.

The swelling pressure of a gel (and presumably of a structural system like protoplasm) is related to vapor pressure and molecular weight according to the following equation (**64**, p. 471):

$$P = -\frac{RT}{MV_o} ln\, h$$

in which $P$ is the swelling pressure, $R$ the gas constant, $T$ the absolute temperature, $M$ the molecular weight, $V_o$ the specific volume of the liquid, and $h$ the relative vapor pressure (the ratio of the vapor pressure of the gel to that of the pure solvent). Hence, if an agent decreases the molecular weight of cellular proteins or the vapor pressure of the protoplasm, the swelling pressure will increase. Accordingly, if water be present and the walls are plastic, cells may enlarge. The vapor pressure of protoplasm may be lowered when cellular proteins dissociate through the splitting of proteins, the liberation of ions, sugars, and other substances from their previous combinations, and by the disengagement and activation of hydrolyzing enzymes.

The diminution of the molecular weight of proteins, conditioned by auxins and other agents, may greatly augment the protoplasmic swelling pressure. According to the equation, if the molecular weight be halved the swelling pressure will be doubled; if quartered, quadrupled; and so on. That proteins may be strikingly dissociated is demonstrated by the work of HAND (**22**), who noted that the particle weight of urease was reduced from 700,000 to 17,000 by urea. If such a decided dissociation occurred when cellular proteins were disaggregated by auxins or other agents, the swelling pressure would be increased more than forty-one times.

The previously presented data demonstrate that in many instances cell enlargement is correlated with dissociation. In some cases the relationship may or may not hold, particularly where positive curvatures were noted and when the viscosity was decreased about equally on both sides. In such instances, however, it is conceivable that at the times the experiments were performed both sides were growing at the same rate.

In some circumstances other substances which dissociate cellular proteins also increase growth. Of the agents which condition a dissociation, the following may at least initially accelerate growth: anesthetics (**16, 39**), carbon dioxide (**57**), disease (**24**), alternating currents (**39**), localized injury (**57**), and X-irradiation (**15**). Under certain conditions the parallelism between growth and the state of cellular proteins is striking. For example, PFEFFER (**57**), GASSNER (**17**), and HARTMANN (**23**) reported a greater growth on the positive side when plants were weakly stimulated by an electrical current and a greater growth on the negative side when more

strongly stimulated. These results parallel the effects that an electrical current has on the dissociation of cellular proteins as evidenced by viscosity changes. NORTHEN (**51**) noted that with short exposures to a current the decrease in structural viscosity was more extensive on the positive side than on the negative, whereas with longer exposures a decrease was observed on the negative side and an increase on the positive side. Hence it seems that the dissociations conditioned by a direct current, auxins, or other agents may at times condition an accelerated growth. When walls are sufficiently plastic, such increased growth may result from an increased protoplasmic swelling, a concept previously advanced by STRUGGER (**72**). However, auxins not only condition an increased swelling pressure, but—as HEYN (**26**) has demonstrated—they also condition an increase in the plasticity of cell walls. Such increased plasticity may or may not result through the intermediation (dissociation) of protoplasm.

Studies on the reactivity of cells to growth substances suggest that dissociation-association reactions of cellular proteins play a role in growth, although not necessarily a primary one in all instances. For example, BOYSEN-JENSEN (**9**) suggested that the positive phototropic curvatures of *Raphanus* hypocotyls were conditioned in part by a greater reduction in the reactivity of cells on the illuminated side than on the shaded side. The studies of WEBER (**80**), who noted that the viscosity of protoplasm was much higher in light than in darkness, suggest that the greater reactivity is conditioned by a greater dissociation of cellular proteins on the shaded side, on which side the swelling pressure would accordingly be greater.

The reactivity to unequal illumination may be modified by many substances. RINA and ARDUINO-JOLANDA (**63**) noted that plants watered with dilute solutions of copper sulphate, caffeine, quinine chlorhydrate, strychnine, ethyl alcohol, and nicotine showed a greater response to unilateral lighting than did plants watered with distilled water. RICHTER (**62**) observed that plants exposed to an atmosphere containing ether exhibited a greater heliotropic response than did control plants. In certain concentrations and with some exposures, caffeine (NORTHEN, unpublished observations), copper chloride (**2**), ethyl alcohol (**25, 50**), and ether (**13, 50**) condition a decrease in protoplasmic viscosity. One interpretation of the increased sensitivity may be as follows: The concentrations of the applied reagents were not high enough in the reacting cells to result in marked dissociation of the cellular proteins, although they were sufficient to condition the formation of labile proteins, which would be more readily dissociated by auxin (which accumulates on the shaded side) than native proteins.

According to this interpretation the sensitivity of a tissue is increased by agents which transmute native to labile proteins. This concept may also explain the data of HITCHCOCK and ZIMMERMAN (**29, 30**). They noted that auxin preparations which contained 0.01 per cent ethyl alcohol were more effective in inducing root

formation than similar preparations not containing alcohol; they found furthermore that greater than additive effects were obtained with mixtures composed of one active root-inducing substance and one or more substances of lower or no activity. In these instances the dilute alcohol and the substances of lower or no activity may have conditioned the formation of more sensitive labile proteins.

When growth is augmented the energy requirements are increased. Such requisites are generally fulfilled by an acceleration of respiration. BONNER (**8**) demonstrated that cell elongation is closely correlated with respiration. THIMANN (**74**) obtained evidence which associated the action of auxins with an oxidation reaction. Hence it seems that respiration is accelerated by the same structural alterations as those which augment swelling pressure.

When cellular proteins dissociate, disulphide linkages are broken, with resulting increase in the number of sulfhydryl (SH) groups. Such groups may actuate many respiratory enzymes. RAPKINE (**60**) noted that the enzyme which catalyzed the oxidation reduction between triose phosphate and pyruvate was active only when sulfhydryl groups were present. GILL and LEHMANN (**18**) quoted the work of QUASTEL and WHEATLEY and that of RUNNSTRÖM and SPERBER, which indicated that the respiration of yeast was increased by SH-glutathione and cysteine. Moreover, they noted that reducing compounds and agents which increased the content of SH groups in the medium augmented Robison ester formation.

Several investigators have found that dehydrogenases are actuated by SH groups (**61, 32**). That dehydrogenase activity is closely related to growth is evident from the investigation of COMMONER and THIMANN (**12**), who observed that dehydrogenase inhibitors restrained the growth of *Avena* coleoptiles. Furthermore, it is possible that during dissociation activators other than SH groups may be disengaged (such as ions), that substrate molecules or the enzymes themselves may be disunited and thereby increase the respiratory rate.

The quickened rate of respiration, conditioned by auxins or other dissociating agents, may in part be caused by an increase in the amount of respirable substrate as a result of polysaccharide hydrolysis. Carbohydrases as well as respiratory enzymes are actuated by sulfhydryl groups, formed when cellular proteins dissociate. MICHAELIS and RUNNSTRÖM (**46**) noted that the glucolytic enzyme of muscle extract was activated by SH groups. ITO (**35**) reported that the hydrolytic activity of $\alpha$-amylase, $\beta$-amylase, and sucrase was accelerated by reduction and retarded by oxidation. In addition to the activation of carbohydrases by such reducing groups as sulfhydryl, the dissociation may result in the disengagement of enzymes from previous combination. KURSSANOV (**37**) states that enzymes are partitioned in the cell between the structural elements and the aqueous solution. In solution the enzymes hydrolyze and, when adsorbed, synthesize. Furthermore, he believes that changes in the physico-chemical conditions in the cell may modify

the ratio. When cellular proteins dissociate, carbohydrases may accumulate in the aqueous phase, thus conditioning hydrolysis. Hence it is likely that those structural alterations which condition increased swelling pressure and respiration also condition increased polysaccharide hydrolysis.

Growth substances often bring about the formation of roots, and such formation may be conditioned by the dissociation of cellular proteins. The appearances of cells and their activities are regulated primarily by the protoplasmic patterns within the cells. Hence, if cells which normally would not divide and develop into root cells are induced to do so, the protoplasmic patterns in the normal cells have been altered. Before a modified pattern can be produced the cellular proteins must be dissociated. Then the products of dissociation may be rearranged (reassociated) to form a dissimilar pattern, one that will function differently. This concept is in complete harmony with recent biochemical data. TISELIUS and HORSFALL (**78**) noted that after hemocyanins from different species were dissociated, the reassociated proteins differed from the original ones. PEDERSEN (**56**) determined the presence of an interaction between components in protein mixtures. Such interactions gave rise to new molecular types.

In the development of the abscission layer a series of protoplasmic changes occur. The dissociation of protoplasm by auxins will interrupt such orderly changes and thereby delay the formation of the layer.

Auxins, like many other substances, may stimulate, restrain, or kill, the end reaction depending upon concentration and time. THIMANN (**74**) has diagrammed the relationship of concentration to effect for stems, roots, and buds. In general, higher concentrations are required for retardation than for stimulation and still higher for death. The dissociation-association concept, outlined in the early part of this paper, adequately shows the interrelationship between death, retardation, and stimulation.

In general, actively growing tissues are more sensitive to potentially lethal agents than are dormant or nongrowing tissues. In actively growing tissues the cellular proteins are usually somewhat dissociated, whereas in dormant tissues the proteins are not. Dissociated proteins may be denatured more readily than native ones. LUNDGREN and WILLIAMS (**43**) found that dissociation and denaturation were closely related. When cellular proteins denature, death is near. To be sure, the sensitivity of tissues is closely related to water content. But such sensitivity is probably primarily conditioned by the state of the cellular proteins. That is, an increase in the water content of a cell may condition some dissociation with a concomitant increased sensitivity, respiration, carbohydrate hydrolysis, and swelling pressure. That dilution may condition a dissociating of cellular proteins is evident from the work of SVEDBERG (**73**), who noted that high dilution caused the dissociation of *in vitro* proteins.

**Summary**

1. The centrifuge method was used to determine protoplasmic viscosity in cortex cells (and in some instances in ray and pith cells) of bean petioles and stems which had been daubed with lanolin preparations of indole-3-acetic acid, indole-3n-propionic acid, and α-naphthalene acetic acid.

2. Unilateral applications of the growth substances initially conditioned negative curvatures, in which instances the protoplasmic viscosity was lower in cells on the faster growing sides of the petioles and stems than in cells on the slower growing sides, and likewise lower than in control petioles or stems. When subsequent positive curvatures resulted, the viscosity was decreased about equally on the treated and untreated sides, except in the case of petioles treated with a paste containing 100 mg. of indole-3n-propionic acid per gram of lanolin, in which instance the viscosity was lower on the untreated sides.

3. Applications of indole-3n-propionic acid or α-naphthalene acetic acid to the tops of decapitated plants resulted in a decrease in the structural viscosity of protoplasm in cells below the cut surfaces.

4. In part at least, the decreases in structural viscosity were conditioned by dissociations of cellular proteins; that is, by the splitting of proteins into molecules one-half, one-fourth, one-eighth, etc., that of the original ones.

5. Biochemical and thermodynamical evidence is introduced which suggests that such dissociations condition increased protoplasmic swelling pressure, respiration, and polysaccharide hydrolysis, as well as altering development.

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LITERATURE CITED

1. ALEXANDER, T. R., Carbohydrates of bean plants after treatment with indole-3-acetic acid. Plant Physiol. **13**:845–858. 1938.
2. ANGERER, C. A., The effect of salts of heavy metals on protoplasm. I. The action of cupric chloride on the viscosity of sea-urchin eggs. Jour. Cell. Comp. Physiol. **10**:183–197. 1937.
3. ———, The effect of electric current on the relative viscosity of sea-urchin egg protoplasm. Biol. Bull. **77**:399–406. 1939.
4. AUDUS, L. J., Mechanical stimulation and respiration rate in the cherry laurel. New Phytol. **34**:386–402. 1935.
5. BEAL, J. M., Effect of indoleacetic acid on thin sections and detached segments of the second internode of the bean. BOT. GAZ. **102**:366–372. 1940.
6. BELLUCCI, B., Beiträge zur Frage des Angriffspunktes der Strahlen an der lebenden Materie. Strahlentherapie **65**:547–568. 1939.
7. BLUM, J. L., Responses of sunflower stems to growth-promoting substances. BOT. GAZ. **102**:737–748. 1941.
8. BONNER, JAMES, The action of the plant growth hormone. Jour. Gen. Physiol. **17**:63–76. 1933.

9. BOYSEN-JENSEN, P., Growth regulators in the higher plants. Ann. Rev. Biochem. **7**:513–528. 1938.
10. BROOKS, M. M., Comparative studies on respiration. XV. The effect of bile salts and saponin upon respiration. Jour. Gen. Physiol. **3**:527–532. 1921.
11. CHAMBERS, L. A., The effect of intense sound waves on some biologically important chemical reactions. Amer. Jour. Med. Sci. **190**:857. 1935.
12. COMMONER, B., and THIMANN, K. V., The relation between growth and respiration in the *Avena* coleoptile. Jour. Gen. Physiol. **24**:279–296. 1941.
13. DAUGHERTY, K., The action of anesthetics on amoeba protoplasm. Physiol. Zoöl. **10**:473–483. 1937.
14. DELAGE, Y., Experimental parthenogenesis by electric charges. Compt. Rend. **147**:553–557. 1909. (Chem. Abst. **3**:193. 1909.)
15. DIETZ, T. J., Volume increase of bacteria from X-ray irradiation. Radiology **24**:31–38. 1935.
16. GAIN, E., Effets de l'anesthésie sur la croissance d'*Allium:* observation du choc anesthésique. Compt. Rend. Soc. Biol. **93**:763–764. 1925.
17. GASSNER, G., Über die Untersuchungen von J. Zeidler zur Frage des Galvanotropismus der Wurzeln. Ber. deutsch. Bot. Ges. **41**:17–19. 1923.
18. GILL, P.M., and LEHMANN, H., Some factors influencing the formation of Robison ester from glycogen and inorganic phosphate in muscle extract. Biochem. Jour. **33**:1151–1170. 1939.
19. GODWIN, H., The effect of handling on respiration of cherry laurel leaves. New Phytol. **34**: 403–406. 1935.
20. GREENLEAF, W. H., Induction of polyploidy and sterility in amphidiploids by hetero-auxin treatment. Amer. Jour. Bot. **26**:673. 1939.
21. HAAS, A. R. C., Effect of anesthetics upon respiration. BOT. GAZ. **67**:377–404. 1919.
22. HAND, D. B., Molecular weight and association of the enzyme urease. Jour. Amer. Chem. Soc. **61**:3180–3183. 1939.
23. HARTMANN, H., Reaktionen von Koleoptilen und Wurzeln im Elektrischen Feld. Beitr. Biol. Pflanzen (Cohn) **19**:287–333. 1932.
24. HEALD, F. D., Manual of plant diseases. New York. 1926.
25. HEILBRUNN, L. V., An outline of general physiology. Philadelphia. 1937.
26. HEYN, A. N. J., The physiology of cell elongation. Bot. Rev. **6**:515–574. 1940.
27. HINDERER, T., The alteration of hereditary tendencies under the action of carbon dioxide. Arch. Entwickl. Organ. **38**:187–209; 364–401. 1914. (Chem. Abst. **8**:1310. 1914.)
28. HITCHCOCK, A. E., and ZIMMERMAN, P. W., Use of physiological responses for determining absorption and transport of synthetic growth substances added to soil. Contr. Boyce Thompson Inst. **7**:447–476. 1935.
29. ———, The use of green tissue test objects for determining the physiological activity of growth substances. Contr. Boyce Thompson Inst. **9**:463–518. 1938.
30. ———, Effect obtained with mixtures of root inducing and other substances. Contr. Boyce Thompson Inst. **11**:143–160. 1940.
31. HOPKINS, E. F., Variation in sugar content in potato tubers caused by wounding and its possible relation to respiration. BOT. GAZ. **84**:75–88. 1927.
32. HOPKINS, F. G., MORGAN, E. J., and LUTWAK-MANN, C., The influence of thiol groups in the activity of dehydrogenases. II. Biochem. Jour. **32**:1829–1847. 1938.
33. HOWARD, W. L., Physiological changes accompanying breaking of the rest period. 5th report. Missouri Agr. Exp. Sta. Res. Bull. 21. 1915.

34. IRWIN, M., Comparative studies on respiration. VI. Increased production of carbon dioxide accompanied by decrease of acidity. Jour. Gen. Physiol. **1**:399–403. 1919.
35. ITO, R., Effects of reduction and oxidation on the reversible action of α–amylase, β-amylase, amylosynthease, sucrase, and urease. Sitzber. freien arztl. Ver. Med. Fakultät Kaiserl. Kyūsyū Univ. Hukuoka (266 Sitzung 16 Dez., 1938); Hukuoka Acta Med. **32**:88. 1939. (Chem. Abst. **35**:5917. 1941.)
36. KRAUS, E. J., BROWN, NELLIE A., and HAMNER, K. C., Histological reactions of bean plants to indoleacetic acid. BOT. GAZ. **98**:370–420. 1936.
37. KURSSANOV, A. L., Untersuchung enzymatischer prozesse in der Lebenden Pflanze. Advances Enzymol. **1**:329–370. 1941.
38. KÚTHY, A. VON, The effect of surface-active substances on gelatins. II. Gel-sol transformation. Biochem. Zeitschr. **244**:331–336. 1932.
39. LAIBACH, F., Interferometrische Untersuchungen an Pflanzen. II. Die Verwendbarkeit des Interferometers in der Pflanzenphysiologie. Jahrb. wiss. Bot. **76**:218–282. 1932.
40. LEBEDEV, A., Concerning the action of the alternating current upon the hydrolyzing characteristic of diastase and mineral acids. Biochem. Zeitschr. **9**:392–402. 1909. (Chem. Abst. **3**:188. 1909.)
41. LLOYD, D. J., Protein structure and water absorption. Jour. Phys. Chem. **42**:1–10. 1938.
42. LOEB, J., On the fertilization and cytolytic effect of soap. Proc. Soc. Exp. Biol. Med. **6**: advance sheets. 1909.
43. LUNDGREN, H. P., and WILLIAMS, J. W., Ultracentrifugal analysis and stability in protein systems. Jour. Phys. Chem. **43**:989–1002. 1939.
44. MATHEWS, A. P., Division of unfertilized eggs. Amer. Jour. Physiol. **4**:343–347. 1900.
45. MCCLENDON, J. F., On artificial parthenogenesis of the sea-urchin egg. Science **30**:454–455. 1910.
46. MICHAELIS, L., and RUNNSTRÖM, J., Inactivation and regeneration of the glucolytic enzyme system of muscle extract. Proc. Soc. Exp. Biol. Med. **32**:343–349. 1934.
47. MITCHELL, J. W., Effect of naphthalene acetic acid and naphthalene acetamide on some nitrogenous and carbohydrate constituents of bean plants. BOT. GAZ. **101**:688–699. 1940.
48. MITCHELL, J. W., KRAUS, E. J., and WHITEHEAD, MURIEL R., Starch hydrolysis in bean leaves following spraying with alpha-naphthalene acetic acid emulsion. BOT. GAZ. **102**: 97–104. 1940.
49. MUIR, R. M., Effect of bile salts and oleates on the structural viscosity of protoplasm. BOT. GAZ. **102**:357–365. 1940.
50. NORTHEN, H. T., Protoplasmic structure in *Spirogyra*. III. Effects of anesthetics on protoplasmic elasticity. BOT. GAZ. **100**:238–244. 1938.
51. ———, Studies on the protoplasmic nature of stimulation and anesthesia. II. Plant Physiol. **15**:645–660. 1940.
52. ———, Effects of protein dissociating agents on the structural viscosity of protoplasm. Biodynamica **3**:10–27. 1940.
53. NORTHEN, H. T., and MACVICAR, R., Effect of X-rays on the structural viscosity of protoplasm. Biodynamica **3**:28–32. 1940.
54. NORTHEN, H. T., and NORTHEN, R. T., Studies of protoplasmic structure in *Spirogyra*. II. Alterations of protoplasmic elasticity. Protoplasma **31**:9–19. 1938.
55. NORTHROP, J. H., The swelling of isoelectric gelatin in water. Jour. Gen. Physiol. **10**:893–904. 1927.
56. PEDERSEN, K. O., Ultracentrifugal studies on protein mixtures. Compt. Rend. Trav. Lab. Carlsberg, Ser. Chim. **22**:427–433. 1938.

57. PFEFFER, W., Physiology of plants (Engl. trans. by A. J. EWART). Oxford. 1905.
58. PRATT, R., Influence of indole-3-acetic acid on the respiration and growth of intact wheat seedlings. Amer. Jour. Bot. **25**:389–392. 1938.
59. ———, Respiration of wheat infected with powdery mildew. Science n.s. **88**:62–63. 1939.
60. RAPKINE, L., Sulfhydryl groups and enzymic oxidation-reduction. Biochem. Jour. **32**:1729–1739. 1938.
61. RAPKINE, L., and TRPINAC, P., Role of sulfhydryl groups of proteins in the activity of dehydrogenases. Compt. Rend. Soc. Biol. **130**:1516–1518. 1939.
62. RICHTER, O., Increase in the heliotropic sensitiveness of seedlings due to narcotics. Sitzb. Akad. Wiss. Wien, Math-Naturw. **121**:1183–1228. 1914. (Chem. Abst. **8**:957. 1914.)
63. RINA, G., and ARDUINO-JOLANDA, R., Contributo allo studio dell'eliotropismo nelle piante. L'azione di diverse sostanze eccitanti sopra di esso. Natura (Milano) **18**:1–27. 1927. (Biol. Abst. **3**:830. 1929.)
64. SCHMIDT, C. L. A., The chemistry of the amino acids and proteins. Baltimore. 1938.
65. SHARP, L. W., Introduction to cytology. New York. 1934.
66. SHULL, C. A., and MITCHELL, J. W., Stimulative effects of X-rays on plant growth. Plant Physiol. **8**:287–296. 1933.
67. SNOW, R., Activation of cambial activity by pure hormones. New Phytol. **34**:347–360. 1935.
68. SÖDING, H., Über das Wachstum der Infloreszenzschäfte. Jahrb. wiss. Bot. **77**:627–656. 1933.
69. SOKOLOV, I. I., Structure and physicochemical properties of "disaggregated" gelatin. Colloid Jour. (U.S.S.R.) **4**:533–549. 1938. (Chem. Abst. **33**:6120. 1939.)
70. SØRENSEN, S. P. L., The constitution of soluble proteins (reversibly dissociable component systems). Compt. Rend. Trav. Lab. Carlsberg **18**:1–124. 1930.
71. STEINHARDT, J., Total dissociation of horse hemoglobin. Nature **138**:800–801. 1936.
72. STRUGGER, S., Beiträge zur Physiologie des Wachstums. I. Zur protoplasma-physiologischen Kausalanalyse des Streckungswachstums. Jahrb. wiss. Bot. **79**:406–471. 1934.
73. SVEDBERG, T., Protein molecules. Chem. Rev. **20**:81–98. 1937.
74. THIMANN, K. V., Hormones and the analysis of growth. Plant Physiol. **13**:437–449. 1938.
75. THIMANN, K. V., and BONNER, J., Plant growth hormones. Physiol. Rev. **18**:524–553. 1938.
76. THORNTON, N. C., Carbon dioxide storage. V. Breaking the dormancy of potato tubers. Contr. Boyce Thompson Inst. **5**:470–480. 1933.
77. ———, Carbon dioxide storage. VIII. Chemical changes in potato tubers resulting from exposure to carbon dioxide. Contr. Boyce Thompson Inst. **7**:113–118. 1935.
78. TISELIUS, A., and HORSFALL, F. L., Mixed molecules of hemocyanins from two different species. Jour. Exp. Med. **69**:83–101. 1939.
79. TURFITT, G. E., A new method for the determination of phytohormone activity. Biochem. Jour. **35**:237–244. 1941.
80. WEBER, F., Plasmolysezeit und Lichtwirkung. Protoplasma **7**:256–258. 1929.
81. ZIMMERMAN, P. W., and HITCHCOCK, A. E., Experiments with vapors and solutions of growth substances. Contr. Boyce Thompson Inst. **10**:481–508. 1939.

# RESISTANCE OF CUCUMBER SEEDLINGS TO DAMPING-OFF AS RELATED TO AGE, SEASON OF YEAR, AND LEVEL OF NITROGEN NUTRITION[1]

T. T. McCLURE AND W. R. ROBBINS

(WITH TWENTY FIGURES)

## Introduction

This paper presents the results of an investigation on the manner in which cucumber seedlings show different degrees of resistance to damping-off after being inoculated with a form of *Pythium*. The lots of seedlings tested differed with regard to the level of nitrogen in the nutrient solutions with which they were supplied and with regard to age and season of the year in which they were grown. Inoculations of seedlings with the fungus culture were made at frequent intervals from May to July, 1937, and from November to April, 1938. Structural modifications of tissues of the seedlings associated with the relative resistance to the infection were studied.

Damping-off diseases are generally considered more likely to occur in seedlings than in older plants, and resistance to infection by the causal fungus organisms increases as seedlings grow older. The fungi causing damping-off have an exceedingly wide host range and can grow parasitically or saprophytically. Because of the wide range of environmental conditions under which these organisms may develop, a number of environmental factors have been associated with resistance or susceptibility to infection. It has been noted that high temperature, high humidity (25), high soil moisture (4), and deficiency in the light supply (1) all favor development of the diseases. The generalization has been made that seedlings in a "weakened" condition are more susceptible than are more vigorously growing ones (8), although this suggestion does not throw much light on the nature of the resistance.

Regarding the influence of the mineral nutrition of the host on susceptibility to infection, LAURENT (according to JONES, 12) concluded that field use of nitrogenous fertilizers predisposed foliage and tubers of potato to infection by *Phytophthora* but COOK (6) found that tomato plants grown with low nitrate supply were unusually susceptible to infection by *Fusarium lycopersici*.

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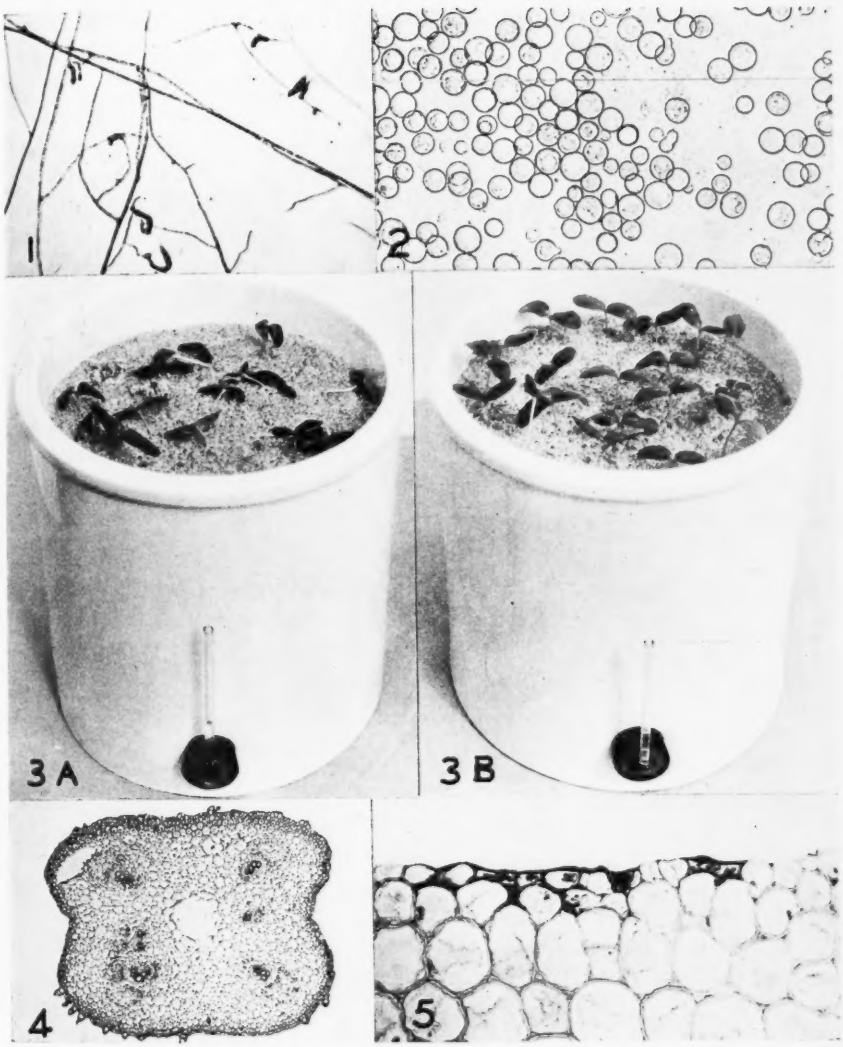



FIGS. 1–5.—Fig. 1, allantoid bodies *Pythium splendens*. Fig. 2, conidia in Mehrlich's liquid medium. Fig. 3, cucumber seedling inoculated at 6 days of age: *A*, with deficient nitrogen; *B*, with adequate nitrogen. Fig. 4, transection of hypocotyl of a 6-day healthy seedling. Fig. 5, transection of scar formation; hypocotyl of resistant seedling.

The resistance of plants to infection by pathogenic fungi has often been ascribed to some morphological feature of the plant tissue. HARTLEY (9) thought that the spread of *Pythium debaryanum* in conifer tissues was hindered by thick cell walls, and HEALD (10) found an inverse correlation between the thickness of parenchyma walls and the spread of *Phytophthora infestans* in potato tubers. In some host plants the progress of an infecting fungus may be greatly retarded or stopped by special growth reactions of neighboring cells. BRAUN (3) found that the resistance to the spread of *Pythium complectens* in geranium cuttings was associated with the formation of a corky layer in advance of the fungus. TISDALE (23) observed a similar walling-off in *Linum* when invaded by *Fusarium*, but ULLSTRUP (24) found no such barrier to the advance of *Fusarium* in China aster wilt. CONANT (5) reported a close correlation between the resistance of tobacco seedlings to infection by *Thielavia basicola* and cork formation in the tissues underlying the lesions.

## Experimental methods

CULTURE OF HOST PLANTS.—Cucumber (*Cucumis sativus* Linn.) was chosen for this study because this species is very susceptible to infection by Pythiaceous

TABLE 1

COMPOSITION OF NUTRIENT SOLUTIONS EXPRESSED AS VOLUME MOLECULAR CONCENTRATIONS OF SALTS*

| SOLUTION | $KH_2PO_4$ | $CaCl_2$ | $Ca(NO_3)_2$ | $MgSO_4$ | $(NH_4)_2SO_4$ |
|---|---|---|---|---|---|
| Complete | 0.00105 | ..... | 0.00365 | 0.00355 | 0.0007 |
| Without nitrogen | 0.00105 | 0.00365 | ..... | 0.00355 | ..... |

* Iron, boron, and manganese used in each solution in concentrations of 1, ½, and ½ p.p.m., respectively.

fungi (7, 22) and because the seedlings are readily handled in experimentation of this kind. Cucumber seeds of the variety Improved Long Green were surface treated for a few minutes with 0.1 per cent aqueous solution of mercuric chloride, after which they were rinsed with sterile water and allowed to germinate between moist sheets of sterile blotting paper in covered glass crystallizing dishes at 24° C. After a germination period of 30 hours, apparently healthy seedlings with radicles protruding about 1 mm. were selected and planted 1 cm. deep in quartz sand held in 2-gallon glazed crocks (fig. 3) with bottom drainage.

Each culture was supplied with about 1 liter of nutrient solution per day, according to the constant drip procedure of SHIVE and STAHL (20). Some cultures received a complete nutrient solution; others received a solution lacking nitrogen. These solutions were modifications of JONES and SHIVE'S (13) four-salt solution $T_1R_1C_5$, except that the osmotic concentration was equivalent to about 0.5 atmos-

phere. The composition of these solutions is given in table 1. The seedlings supplied with the complete solution had an adequate supply of nitrogen, whereas in the other treatment the seedlings received no nitrogen except that which was derived from the seeds or present as unavoidable impurities introduced with the use of analytically pure nutrient salts of the grade Baker's Analyzed.

TABLE 2

INCIDENCE OF INFECTION IN SEEDLINGS GROWN WITH ADEQUATE AND WITH INADEQUATE NITROGEN SUPPLY AND INOCULATED ON HYPOCOTYL WITH PYTHIUM AT VARIOUS AGES AND AT VARIOUS TIMES OF YEAR

| Date | | Age (days) at start of inoculation (and no. of days in inoculation period) | Incidence of infection (killed or scarred) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Adequate nitrogen | | | Deficient nitrogen | | |
| Culture started | Inoculation started | | No. inoculated | Killed (%) | Scarred (%) | No. inoculated | Killed (%) | Scarred (%) |
| 5/5/37 | 5/18/37 | 13 (7) | 15 | 0 | 27 | 18 | 0 | 61 |
| 5/12 | 5/18 | 6 (7) | 18 | 0 | 61 | 19 | 95 | 5 |
| 6/9 | 6/22 | 13 (4) | 20 | 0 | 10 | 20 | 0 | 30 |
| 6/9 | 6/22 | **13 (4)*** | **10** | **0** | **100** | **10** | **10** | **90** |
| 6/16 | 6/24 | 8 (4) | 20 | 0 | 25 | 20 | 0 | 40 |
| 6/22 | 6/28 | 6 (2) | 20 | 100 | 0 | 20 | 100 | 0 |
| 6/30 | 7/8 | **8 (2)** | **10** | **0** | **100** | **9** | **0** | **100** |
| 10/13 | 11/2 | 20 (4) | 16 | 0 | 25 | 16 | 0 | 38 |
| 10/27 | 11/2 | 6 (4) | 20 | 100 | 0 | 18 | 100 | 0 |
| 10/27 | 11/13 | 17 (4) | 20 | 50 | 30 | ..... | ..... | ..... |
| 11/3 | 11/13 | 10 (4) | 20 | 100 | 0 | 20 | 100 | 0 |
| 11/24 | 12/7 | **13 (4)** | **20** | **100** | 0 | ..... | ..... | ..... |
| 12/1 | 12/7 | 6 (4) | 20 | 100 | 0 | ..... | ..... | ..... |
| 12/1 | 12/14 | 13 (4) | 20 | 95 | 5 | ..... | ..... | ..... |
| 12/16 | 1/5/38 | 20 (3) | 31 | 100 | 0 | ..... | ..... | ..... |
| 12/16 | 1/5 | **20 (3)** | **15** | **100** | **0** | ..... | ..... | ..... |
| 12/16 | 1/13 | 28 (2) | 16 | 100 | 0 | ..... | ..... | ..... |
| 2/18/38 | 3/19 | 29 (2) | 10 | 90 | 10 | 10 | 70 | 30 |
| 3/6 | 3/19 | 13 (2) | 19 | 100 | 0 | 20 | 100 | 0 |
| 3/11 | 4/10 | 28 (2) | 9 | 11 | 89 | ..... | ..... | ..... |
| 3/11 | 4/10 | **28 (2)** | **5** | **40** | **60** | ..... | ..... | ..... |
| 3/29 | 4/14 | 16 (2) | 10 | 33 | 67 | 9 | 22 | 78 |
| 3/29 | 4/14 | **16 (2)** | **10** | **50** | **50** | **9** | **67** | **33** |

* Data in heavy type are for tests in which inoculation was applied to fresh wound.

The dates on which the sand cultures were started and the duration of their treatments before the seedlings were inoculated are given in table 2. Each culture was continued through the inoculation period and usually for a few days thereafter, to permit any evidences of infection to become unmistakably apparent. The culture crocks stood on a greenhouse bench during the experiments, except during the winter months when they were inclosed in a special chamber glazed with trans-

parent cellulose acetate film. The temperature in this chamber was kept above 20° C. by thermostatically controlled electric heating cable. The day length and average light intensity varied according to the time of year and variations in diurnal cloudiness.

CULTURE OF FUNGUS.—The *Pythium* used was isolated from lettuce seedlings showing the damping-off disease. It is closely similar to *P. splendens* (3) which grows over a temperature range of 11°–37° C., with an optimum temperature of about 30° C. The H-ion requirements of *P. splendens* are not reported, but the closely related *P. debaryanum* has been found to grow well from pH 5 to 7 (2). The fungus of the present study grew readily on potato agar, rice mush, MEHRLICH's agar, or in MEHRLICH's liquid (14). The inoculum was grown on potato agar or rice mush. The *Pythium* showed strong aerial growth on rice mush, but its aerial growth on 1.5 per cent agar media was much less vigorous. On stiff agar, and when in contact with glass, the laterals were often allantoid in shape (fig. 1). The hyphal diameter was usually about 5.8 $\mu$ in the region where the conidia were borne, varying elsewhere from 1.9 to 8.2 $\mu$. Neither zoospores nor oögonia were seen. The nearly spherical conidia (fig. 2) were profuse, usually terminal, with a diameter of 21.7–48.0 $\mu$, usually about 37 $\mu$.

INOCULATION OF SEEDLINGS.—The technique followed for inoculation was essentially like that described by RATHBUN (18). Similar sized pieces of agar or rice mush about 5 mm. in diameter were cut from a plate culture of the fungus, and one of these was applied against one side of the hypocotyl of each of the seedlings to be inoculated, being left in position until the end of the test in each instance. In most of the tests the inoculum was applied to the hypocotyl about 1 cm. above the sand level and when necessary was supported in this position with a tiny cardboard platform. This technique was necessary since the fungus sometimes grew downward through the sand to the seedling roots when the inoculum rested on the sand surface.

In some instances (as indicated by the data in heavy type in table 2) the inoculation was made by slightly scratching the hypocotyl on one side with a needle and immediately applying the inoculum against this lesion. It was thought that this procedure might facilitate primary infection after inoculation, but the results were not very different from those secured without preliminary wounding. After inoculation, the cultures were covered with bell jars, thus providing humidity to keep the inoculum from drying out.

From ten to twenty seedlings were usually inoculated in each test, and the number subsequently killed by the disease was computed as a percentage of the number inoculated. When the seedlings were not killed, primary infection occurred in many cases but failed to develop beyond a small region close to the place of inoculation. In such cases the primary lesion healed, leaving a notable in-

fection scar which was much more pronounced than the slight scratch resulting from the preliminary wounding. For each test the number of seedlings thus scarred by incipient but abated infection was also computed as a percentage of the number of seedlings inoculated. These two percentages are taken as indices of the degree of susceptibility of the seedlings in each test (table 2). For example, of the fifteen seedlings grown with adequate nitrogen supply that were inoculated on May 18, 1937, none were killed but 27 per cent showed infection scars; while of the eighteen seedlings grown with inadequate nitrogen supply and inoculated at that time, none were killed but 61 per cent showed the scars.

HISTOLOGICAL EXAMINATION OF SEEDLINGS.—Fresh samples of tissue were killed and fixed in Allen's $PFA_3$ solution (**15**) and subjected to reduced pressure. Dehydration was carried out with 50 per cent ethyl alcohol and then with butyl alcohol (**11**). Paraffin sections were cut 8–10 $\mu$ thick. For staining, a combination of safranin and fast green was found useful in the study of seedling tissues generally, but a combination of crystal violet and gold orange was especially valuable in showing sharp differentiation between healthy and diseased tissue, while a combination of magdala red and fast green served for contrast between host and parasite.

Among the microchemical tests the following were used. For starch: iodine-potassium-iodide; for cellulose: (*a*) iodine-sulphuric acid and (*b*) chlor-iodide of zinc; for lignin: (*a*) phloroglucin, (*b*) iodine-sulphuric acid, and (*c*) potassium permanganate;[2] for cuticle and cuticularized walls: Sudan IV; for cuticularized walls only: iodine-sulphuric acid. Except for the test with potassium permanganate, these reagents were prepared and used according to the directions given by RAWLINS (**19**).

## Results

The results of infection of the seedlings of various ages after inoculation are recorded in table 2. Seedlings which succumbed to infection were counted as highly susceptible and were designated killed. Seedlings which after inoculation formed slight but visible scars on the hypocotyl at the point of inoculation were designated scarred. These seedlings, however, were considered resistant to complete infection by the organism if further development of the disease did not occur. Inoculated seedlings counted as completely resistant to infection generally showed very slight scars which, although not visible, became evident upon histological examination. This is shown in figure 5 by the darkened cell walls in the epidermal region of transverse section of the hypocotyl.

From the data presented in table 2 it appears that the relative resistance to infection of seedlings of a given age varies with the season of the year. During the winter months seedlings were susceptible at ages at which during the summer months they were found to be resistant. Thus on May 18 and on June 22, 13-day-

[2] ECKERSON, SOPHIA H., Microchemistry (mimeographed).

old seedlings supplied with adequate nitrogen were entirely resistant to complete infection; whereas on December 14, seedlings of the same age with the same nutrient treatment were 95 per cent susceptible and were killed. For the interval of tests from November 2 until March 19, high susceptibility prevailed for seedlings adequately supplied with nitrogen at ages of 6–20 days. The inoculations of January 13 showed complete susceptibility, amounting to death of 100 per cent of the seedlings as old as 28 days. On April 10, 28-day-old seedlings adequately supplied with nitrogen were only 11 per cent susceptible to infection.

With regard to the nitrogen-deficient seedlings, the meager evidence seems to indicate somewhat similar seasonal trends in the relative susceptibility. During the period from about November 2 to about March 19 these seedlings showed high susceptibility, which, however, decreased during the early summer period of May and June.

When the nitrogen level is considered, seedlings adequately supplied with nitrogen were in most cases more resistant to infection than were those with a deficient nitrogen supply. In those tests where death of the seedling did not occur, the greater initial susceptibility of the low nitrogen seedlings is indicated by the consistently greater percentage of scarred seedlings (table 2). On May 18, for example, 95 per cent of the 6-day-old low nitrogen seedlings were killed, whereas none adequately supplied with nitrogen died. Figure 3*A* shows seedlings with a deficient nitrogen supply succumbing as a result of susceptibility. Figure 3*B* shows the resistance of seedlings adequately supplied with nitrogen.

Although this evidence is not extensive, it may warrant the conclusions: (*a*) that young seedlings were generally less susceptible to infection when grown with adequate nitrogen supply than when grown at a low level of nitrogen nutrition; (*b*) that seedlings of any given age were less susceptible to infection in early summer than in winter; (*c*) that the degree of susceptibility of young seedlings appeared generally to decrease with increasing age; and (*d*) that a high degree of susceptibility apparently persisted to a greater age in winter than in early summer.

That seedlings of similar age were much less resistant in early summer than in winter may safely be attributed to seasonal differences in their metabolism, for it is not reasonable to suppose that the composition of the stored seeds became effectively altered in a few months. Progressive physiological changes taking place with advancing age in the direction of what may be called maturation proceeded more rapidly in early summer than in winter, accounting for the fact that seedlings remained susceptible to infection for a longer time during the winter than during the summer season. Except for the influence of fluctuating cloudiness, the two seasonal factors most directly associated in these experiments with seasonal changes were the changes in length of photoperiod and in light intensity. Both factors affect the assimilation of carbon and thereby the available energy for all

endothermic metabolic plant processes. It was not determined which factor was the more important one concerned with seasonal change in resistance. It might readily be expected that the use of artificial light of suitable quality, intensity, and duration might have rendered the winter-grown seedlings just as resistant as the early summer-grown ones.

Histological examination of the hypocotyls of inoculated seedlings that had survived, and particularly of those which had been wounded at the point of inoculation, indicated that resistance to infection was seldom or never due to failure of the fungus to penetrate the epidermis. In the cases examined there was always some penetration and spread of the fungus from the place of entrance. The spread of the mycelium within the tissue was often very slight (fig. 5), but it was more extensive when clearly apparent infection scars were formed. In instances where seedlings survived, it was clear that some change in the host tissue near the region of primary penetration had occurred, and that such changes were associated with and perhaps the cause of the stopping of the further advance of the parasite.

The pH values of the hypocotyl parenchyma of the seedlings grown with adequate nitrogen supply (pH 5.6–5.8) were found to be generally slightly lower than for those with inadequate nitrogen (pH 5.8–6.0), but these pH values lie within the range for good growth of this *Pythium*. No difference was found between the pH values of hypocotyl parenchyma from very susceptible and from highly resistant seedlings.

Microchemical tests showed accumulations of considerable starch in the hypocotyls of the low nitrogen seedlings but no such starch accumulation in similar tissues of those adequately supplied with nitrogen. The mere presence of starch was not necessarily directly correlated with susceptibility, however, because low nitrogen seedlings were found to contain starch even after they had gained a certain degree of resistance with increase in age. The accumulation of starch in the low nitrogen seedlings was to be expected, since this phenomenon is a common occurrence in many other species of plants, accompanying a low rate of nitrogen assimilation (**16, 17**).

Hypocotyls of highly resistant seedlings were killed by freezing, heating, or treatment with strong alcohol, and after inoculation were found to support vigorous growth of the fungus used in this experiment. Hyphae were found to have penetrated throughout the dead host tissue, thus suggesting that resistance to infection ceases when the hypocotyl is killed and that resistance depends upon the activity of living cells—or at least upon features no longer effective after their death.

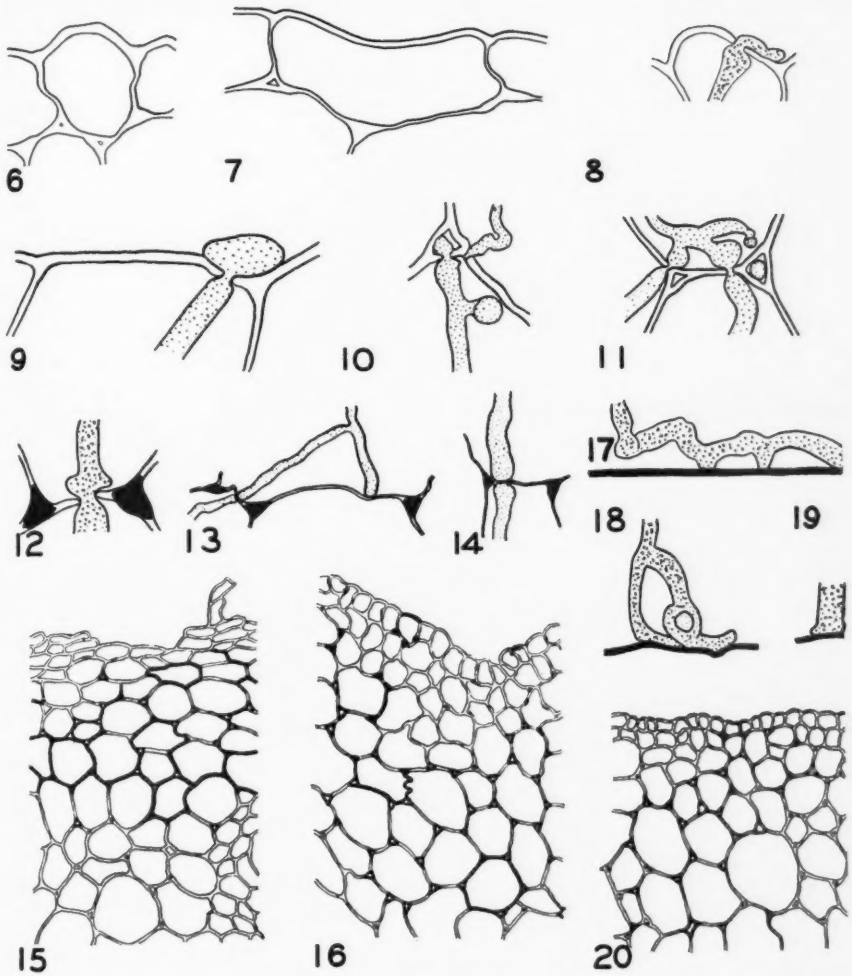
By means of transverse sections cut 1 cm. above the sand level, uninoculated hypocotyls of healthy young seedlings—like those found to be highly susceptible to infection—were compared with uninoculated hypocotyls of older seedlings,

such as those found to be highly resistant. Except for size they were very similar, and every type of tissue present in the older hypocotyls was already present in the younger ones (fig. 4). Average wall thickness in the fundamental tissues was the same in both cases—about 0.5 $\mu$.

The thickness of the outer epidermal walls, including the cuticle, had apparently decreased slightly with increasing age of the seedlings; in the younger seedlings these walls were about 2.0 $\mu$ thick (fig. 6), whereas they were only about 1.5 $\mu$ thick in the older seedlings (fig. 7), perhaps owing to stretching without corresponding thickening. The cuticle was of the same thickness in both cases—about 0.5 $\mu$. The outer cell walls themselves were composed mainly of cellulose but were slightly cuticularized. The cuticle showed no cellulose reaction. Figures 8 and 9 illustrate the penetration of the fungus into epidermal cells of the hypocotyl of a susceptible and of a resistant seedling, respectively. It therefore appeared that the high degree of resistance which characterized the older hypocotyls in contrast to the younger ones was not due to any maturation change in either cuticle or epidermal walls. In resistant hypocotyls, as has been said, hyphae from the inoculum penetrated somewhat beyond the epidermis (figs. 10, 11), even when no obvious scar resulted, and they were found to have advanced through several cell layers in cases where scars were to be found; but in such cases the spread of the initial infection soon ceased. In highly susceptible hypocotyls the mycelium spread rapidly through the fundamental tissue of the host.

Further examination of the diseased seedling tissue indicated the probable means by which resistance to infection occurred. When microchemical tests for lignin were applied to transverse sections cut through the infection scars, lignification was shown to be much more prevalent beneath and around the tissue of the lesion than elsewhere, suggesting that the spread of the fungus might in these cases have been limited by a barrier of lignified cells. In no case were hyphae seen to have penetrated through lignified regions of cell walls, although they did through nonlignified portions (figs. 12–14). It was not unusual to find hyphae growing in contact with lignified regions of walls as if unable to penetrate them (figs. 17–19). The importance of cork formation in the tissues beneath a lesion in tobacco seedlings has been emphasized by CONANT (**5**), who found that such tissue changes occurred more rapidly in varieties highly resistant than in others highly susceptible to infection by *Thielavia basicola*.

The prevalence of lignification in the region adjacent to the scar in the hypocotyl of a resistant seedling grown with adequate nitrogen is shown in figure 15. This extensive lignification, however, indicated in the figure by the blackened portions of the walls, was not accompanied by any thickening of the walls so affected. Figure 16 shows the infected area of the hypocotyl of a young high nitrogen seedling which was in an advanced stage of infection. Lignification of walls was




FIGS. 6–20.—Fig. 6, epidermal cell of 6-day seedling. Fig. 7, of 17-day seedling. Fig. 8, fungus penetration of epidermal cell; hypocotyl of 6-day susceptible seedling. Fig. 9, fungus penetration of epidermal cell of hypocotyl of 30-day resistant seedling. Figs. 10, 11, fungus penetration in parenchymatous tissue. Figs. 12–14, same of unlignified portions of parenchymatous tissue; lignification shown as solid black. Fig. 15, transection of hypocotyl of 30-day resistant seedling grown with adequate nitrogen after wounding and inoculation. Fig. 16, hypocotyl of 7-day seedling susceptible after inoculation; adequate nitrogen. Figs. 17–19, fungus hyphae in contact with lignified cell walls. Fig. 20, hypocotyl of diseased 30-day low nitrogen seedling.

incomplete. Likewise lignification of cell walls in the tissues of low nitrogen seedlings was not so rapid or so complete in response to inoculation as that occurring in the tissues of resistant seedlings adequately supplied with nitrogen. Figure 20 is a transverse section of the hypocotyl of a 30-day-old, low nitrogen seedling susceptible to infection and shows incomplete lignification of cell walls and absence of a continuous lignified barrier. Fungus hyphae, although not shown in this figure, were seen throughout the tissue of this region.

Many observations of this sort led to the conclusion that the resistance which occurred in the hypocotyls of the seedlings was associated with the formation of lignin barriers and that such barriers were of a nature effectually to prevent further spread of the parasite through the host tissues. Whether such lignification barriers constituted the principal cause of resistance to the invasion through the tissues, or were formed concomitantly as a result of other changes in metabolism which rendered the seedlings resistant, cannot be definitely stated. The known fact that lignin is markedly resistant to enzymatic digestion, however, lends support to the belief that the mere development of a well-developed barrier of lignin would be sufficient effectually to limit the rapid spread of this organism. The formation of such lignin barriers in the tissues appeared to be favored by adequate nitrogen nutrition of the seedlings.

No such complete or nearly complete lignification of cell walls of the fundamental parenchyma was seen in sections from uninoculated hypocotyls that had not been wounded; and sections from badly infected hypocotyls, where spread of the fungus had not been restricted, showed little lignification and no such barriers. But sections of hypocotyls wounded with a sterile needle and not inoculated showed pronounced lignification barriers similar to those resulting from limited infection.

It appears that the capacity for this lignification response to fungus infection, a response apparently protective in its results, is related to physiological maturation of the tissues involved and that the hypocotyl tissues at the region of inoculation developed that physiological capacity more rapidly and sooner when the plants were grown with an adequate nitrogen supply and with long photoperiods of high light intensity levels than at a low nitrogen level or with short photoperiods of low light intensity levels.

## Discussion

As indicated in this study, if resistance to infection is associated with and perhaps significantly dependent upon rapid lignification of cell walls surrounding the region of inoculation, it becomes of interest to consider how the factors found to be associated with relative resistance (level of nitrogen nutrition, age of seedlings, and season of year) are related to the rate of cell-wall lignification.

The deposition in cell walls of lignin, a carbohydrate derivative, is dependent

upon the presence of an adequate tissue content of available carbohydrate (21). During the days of low light intensity in the winter season, carbohydrate synthesis is at a relatively low plane. The rate of lignification was found to be low, and likewise the susceptibility to infection during this season of the year was found to be greater than during the late spring or early summer.

With regard to the increasing resistance to infection with increasing age of seedlings, it may be emphasized that—beginning with germination and during early growth—there is at first a decrease in the level of carbohydrate available for growth. This proceeds until, with the increasing efficiency of the photosynthetic mechanism, the seedling becomes independent of stored carbohydrate. This change takes place more rapidly with adequate than with a low light intensity and may account for the increase in resistance with advancing age and the differences in the ages at which the seedlings become resistant at different seasons of the year. In view of this explanation of resistance, it might be questioned why the seedlings grown with a low plane of nitrogen nutrition showed greater susceptibility to infection than those grown with an adequate nitrogen supply.

It is known that with a low level of nitrogen nutrition, carbohydrates accumulate as a result of their restricted use in the formation of organic nitrogenous compounds. On the other hand, lignin, and cellulose as well, are secretion products of protoplasm. When this secreting system is limited in extent or in activity as a result of a low plane of nitrogen assimilation, the rate of lignification may also be retarded as compared with that in tissues where the plane of nitrogen nutrition is on a somewhat higher level. This may therefore account for the relatively greater susceptibility to infection of the seedlings grown with a deficient than with an adequate nitrogen supply.

The particular level of nitrogen nutrition which would be associated with the maximum degree of resistance to infection was not determined. This level would undoubtedly vary from season to season and be dependent upon the several factors which, taken together, affect either the assimilation of nitrogen or the synthesis of carbohydrates and upon which may depend the capacity of tissues to respond to infection and wounding, by the process of cell-wall lignification.

## Summary

Cucumber seedlings were grown in sand culture at high and low levels of nitrogen nutrition during several different seasons of the year. The seedlings were artificially inoculated at different ages with a fungus of the *Pythium* type, causing the damping-off disease, and the relative resistance or susceptibility to infection noted. The more important results are as follows:

1. Young seedlings were more susceptible to infection than older ones.
2. Seedlings grown under the relatively poor light conditions of winter re-

mained susceptible to infection for a longer time than those grown under the good light conditions of late spring and early summer.

3. Seedlings grown with no external supply of nitrogen remained susceptible to infection for a longer time than seedlings supplied with a complete nutrient solution containing nitrogen.

4. Resistance to infection was accompanied by a deposition of lignin in the cell walls of the tissue surrounding the area of infection. Susceptibility to infection was accompanied by the incompleteness or absence of such cell-wall lignification. It is suggested that such lignification, when continuous, may serve as a barrier in preventing spread of the fungus.

5. Deposition of lignin was found to be a function of living parenchymatous cells and occurred in the vicinity of a wound or fungus infection. In a healthy plant these cells did not become lignified.

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## LITERATURE CITED

1. ATKINSON, G. F., Damping-off. Cornell Agr. Exp. Sta. Bull. 94. 1895.
2. ANDERSON, P. J., *Pythium* damping-off and root-rot in the seed bed. Connecticut Agr. Exp. Sta. Bull. 359. 1934.
3. BRAUN, H., Comparative studies of *Pythium debaryanum* and two related species from geranium. Jour. Agr. Res. **30**:1043–1062. 1925.
4. BEATTIE, J. H., The production of cucumbers in greenhouses. U.S. Dept. Agr. Farmers Bull. 1320. 1923.
5. CONANT, G. H., Histological studies of resistance in tobacco to *Thielavia basicola*. Amer. Jour. Bot. **14**:457–780. 1927.
6. COOK, W. S., Relation of nutrition of tomato to disposition to infectivity and virulence of *Fusarium lycopersici*. BOT. GAZ. **98**:647–669. 1937.
7. DRECHSLER, C., The cottony leak of cucumbers caused by *Pythium aphanidermatum*. Jour. Agr. Res. **30**:1035–1042. 1925.
8. DUGGAR, B. M., Fungous diseases of plants. New York. 1909.
9. HARTLEY, C. H., Damping-off in forest nurseries. U.S. Dept. Agr. Bull. 934. 1921.
10. HEALD, F. D., Manual of plant diseases. New York. 1933.
11. JOHANSEN, D. A., Tertiary butyl alcohol methods. Part II. El Palo Alto News **1**:5–6. 1937.
12. JONES, L. R., Disease resistance of potatoes. U.S. Dept. Agr. Bur. Pl. Ind. Bull. 87. 1905.
13. JONES, L. H., and SHIVE, J. W., Influence of ammonium sulphate on plant growth in nutrient solutions and its effect on hydrogen-ion concentration and iron availability. Ann. Bot. **37**:355–377. 1923.
14. MEHRLICH, F. P., Medium for the growth of Pythiaceous fungi. Phytopath. **24**:1127–1128. 1934.
15. MCCLUNG, C. E., Microscopical technique. New York. 1929.
16. NIGHTINGALE, G. T., The nitrogen nutrition of green plants. Bot. Rev. **3**:85–174. 1937.

17. NIGHTINGALE, G. T., SCHERMERHORN, L. G., and ROBBINS, W. R., The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems, and leaves. New Jersey Agr. Exp. Sta. Bull. 461. 1928.
18. RATHBUN, A. E., Methods of direct inoculation with damping-off fungi. Phytopath. 11: 80–84. 1921.
19. RAWLINS, T. E., Phytopathological and botanical research methods. New York. 1933.
20. SHIVE, J. W., and STAHL, A. L., Constant rates of continuous solution renewal for plants in water cultures. BOT. GAZ. 84:317–323. 1927.
21. SMITH, W. W., The course of stone cell formation in pear fruits. Plant Physiol. 10:587–611. 1935.
22. SPARROW, F. K., JR., Observations on the parasitic ability of certain species of *Pythium*. Phytopath. 22:385–390. 1932.
23. TISDALE, W. H., Flaxwilt: A study of the nature and inheritance of wilt resistance. Jour. Agr. Res. 11:573–606. 1917.
24. ULLSTRUP, A. J., Histological studies on wilt of China aster. Phytopath. 27:737–748. 1937.
25. WATSON, B. M., BAILEY, L. H., and SEYMOUR, A. B., Damping-off. Amer. Garden 11: 348–350. 1890.

# PHOTOPERIODIC AFTER-EFFECTS IN SIX COMPOSITES[1]

VICTOR A. GREULACH

(WITH FIVE FIGURES)

## Introduction

Photoperiodic after-effects were discovered in *Cosmos bipinnatus* by GARNER and ALLARD (3) and have since been observed in several other species of composites. BIDDULPH (1) found that when plants of the Klondike variety of *C. sulphureus* were transferred from short to long photoperiods, anomalies developed, including change from opposite to spiral phyllotaxy, elongation and foliation of bracts, abortive buds, twin flower heads, and elongated internodes in the region of the involucral bracts. GARNER and ALLARD did not describe such structures for *C. bipinnatus*. MURNEEK (7, 8) found that when *Rudbeckia* plants were transferred from long to 7-hour photoperiods some plants failed to bloom; some formed the usual type of flower heads (except that these were either sessile or borne on short peduncles, the plants remaining in the rosette condition); and some bore "vegetative flowers" with green petals and more or less vegetative stamens and pistils.

In an effort to determine whether similar photoperiodic after-effects occur in certain other species of composites, experiments were conducted on *Cosmos bipinnatus* Cav., *C. sulphureus* Cav. (Orange Flare variety), *Rudbeckia hirta* L., *Matricaria parthenoides* Desf., *Centaurea cyanus* L., and *Coreopsis tinctoria* Nutt. Both species of *Cosmos* were found by GARNER and ALLARD (3) to be short-day plants. However, the Orange Flare variety of *C. sulphureus*, unlike the Klondike variety, is not strictly a short-day plant. ROBERTS and STRUCKMEYER (12) reported that it acted as a short-day plant in cool temperatures and as an intermediate or neutral plant at higher temperatures, blooming under both long and short photoperiods. The species of *Rudbeckia* have generally been classed as long-day plants, but ROBERTS and STRUCKMEYER (11) found that under low night temperatures *R. laciniata* formed flower buds after a relatively long time. The other three species were reported by POESCH and LAURIE (9) to be long-day plants.

METHODS.—All plants were maintained in soil in porous clay pots. Four or five replications were made of each treatment, a total of 786 plants being used. All seeds germinated, and the seedlings began growth under photoperiods unfavorable for the initiation of flower primordia, that is, under long photoperiods for the two species of *Cosmos* and under short photoperiods for the other four species. Induction treatments were begun 28 days after planting, short induction photoperiods

[1] Papers from the Department of Botany, Ohio State University, no. 436.

being employed for the two species of *Cosmos* and long ones for the other species. After exposure to one induction photoperiod, four or five plants of each species were returned, for the duration of the experiments, to photoperiods not favoring the initiation of floral primordia. A second set of plants of each species was exposed to two, a third set to three, induction photoperiods, and so on, the maximum number being 20 for *Cosmos bipinnatus*, *C. sulphureus*, and *Centaurea cyanus*, and 30 for the other three species. One set of plants of each species was retained under photoperiods unfavorable for the initiation of floral primordia and another set under photoperiods of the length used in the induction treatments, throughout the experiments.

The experiments were conducted at Columbus, Ohio. They were begun May 18 and concluded September 3, 1939, unless otherwise noted. The plants were started in the greenhouse but were kept outside during the major part of the time. The long photoperiods, 15 or more hours, were secured by employing the natural photoperiod, either alone or supplemented with Mazda lamps. The short photoperiods, 9 hours in length, were secured by means of ventilated light-proof cabinets. The temperature differences between the inside and the outside of the cabinets were too small to have any significant influence on the development of the plants. The temperature during the experiments ranged between 60° and 90° F., except for a few brief periods, the mean temperature being 75° F.

## Results

COSMOS BIPINNATUS.—By July 1, 45 days after planting, flower buds were evident on plants exposed to 9 and to 12 or more short photoperiods. Anthesis first occurred on July 9 on one plant exposed to 13 short photoperiods and on one exposed to 14. From then until August 14, one or more plants bloomed daily, with interruptions of only a day or two. Blooming was confined mostly to plants exposed to 12 or more short photoperiods, but single flower heads developed on one plant each in the groups exposed to 5, 6, and 10 short photoperiods, two heads on a plant exposed to 9 short photoperiods, and three on a plant exposed to 8 short photoperiods. Flower buds developed on a number of other plants but failed to open by the termination of the experiment, August 26. Fruits developed on plants in all groups exposed to 12 or more short photoperiods.

At the time of the last transfer from short to long photoperiods there was marked though somewhat uneven gradation in the height of the plants, those exposed to greater numbers of short photoperiods being smaller. Once under long photoperiods, all plants began growing rapidly; but the plants exposed to greater numbers of short photoperiods were smaller at the end of the experiment, not only because of their delayed start but also because their growth was checked by the formation of terminal flower buds.

Reproductive-vegetative interphases, often more extreme than those observed in *C. sulphureus* by BIDDULPH (1), occurred in abundance. The first anomaly to appear was lengthening of the lower cycle of involucral bracts. These are usually 1 cm. or less in length in this species, but bracts as long as 9 cm. were formed. The longer bracts were often highly lobed and even foliaceous, but simple bracts 5–6 cm. long were not uncommon. Although there was little relation between maximum bract length and number of short photoperiods to which the plants were exposed, both the average and minimum bract lengths were less in the plants exposed to greater numbers of short photoperiods.

FIG. 1.—Representative floral modifications in *Cosmos bipinnatus*, August 1. Numbers refer to number of short photoperiods to which plants had been exposed. From left to right the specimens represent: flower head with elongated bracts (one separated from the others by stem elongation); two with elongated bracts in a whorl some distance below the head, bracts of first one being incised; bracts elongated but not separated from head; bracts elongated and scattered, with reversion to vegetative growth; bracts only slightly elongated and in usual position.

In about 60 per cent of the plants, internodal elongation occurred between the flower head and the lower cycle of involucral bracts. This occurred later than bract elongation and only below heads with elongated bracts. The length of this internode varied from less than 1 cm. to 27 cm. At first such elongation occurred only in plants exposed to 9 or fewer short photoperiods, but eventually was found in the plants exposed to more than 9 short photoperiods. In some plants the bracts were in a perfect whorl (figs. 1, 2); in others only one or a few bracts were separated from the rest (fig. 2); in others stem elongation occurred between all bracts, resulting in a spiral arrangement. Some of the bracts were decurrent, and later in the experiment many divided bracts were formed. The earlier ones had only a few

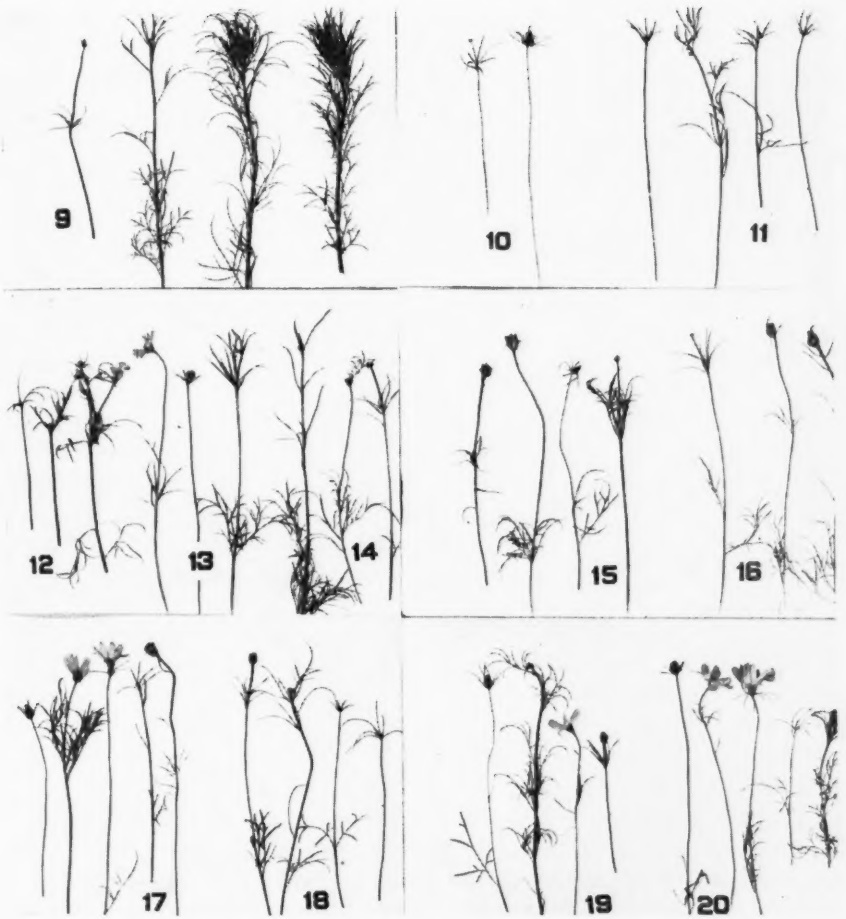



FIG. 2.—Representative floral modifications in *Cosmos bipinnatus*, August 17. Numbers indicate number of short photoperiods to which plants had been exposed. Elongated bracts shown in all the groups: some are adjacent to flower bud or head (11, two to right in 18-day group), some are arranged in whorl some distance below it (9, 10, 15, 19), and some are arranged spirally or irregularly spaced (9, 11, 14, 16). While many bracts were entire (10, 11, 18), others became incised and more or less foliaceous (9, 15, 17, 19). Some flower heads were asymmetrical and distorted (13, 17), and some flower buds were abortive (11, 14). In a few plants reversion to vegetative growth occurred (9).

lobes, but later many foliaceous bracts—resembling ordinary cosmos leaves in the complexity of their incision—were formed.

Small abortive flower buds, some barely visible, developed as the plants grew older. These were almost always subtended by long simple bracts not separated from the stem by internodal elongation (fig. 2). There were also some very small flower heads, or heads containing only a few ray flowers, some of which were foliaceous. There were no twin flower heads such as reported by BIDDULPH for *C. sulphureus*.

Actual resumption of vegetative growth occurred in several plants from terminal flower buds after the lower cycle of bracts had been formed, these usually

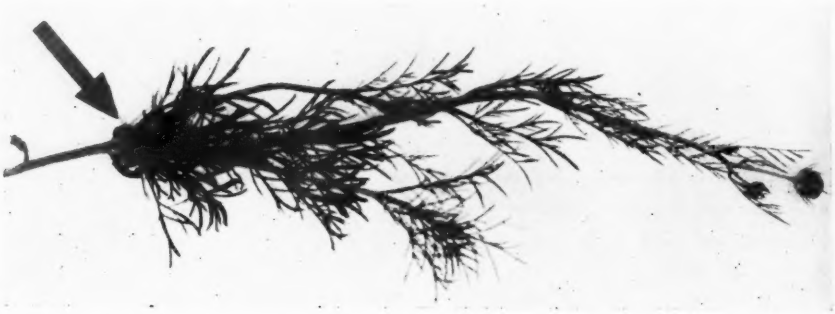


FIG. 3.—Portion of *Cosmos bipinnatus* plant, August 17, which had been exposed to 5 short photoperiods and on which a flower bud began forming at point where lowest bracts are located. Arrow points to single pink petaloid structure which formed before reversion to vegetative development. Later a reversion to reproductive development occurred, two flower buds lacking the lower set of bracts forming. One of these bloomed later.

being elongated and compound. The new stems had unusually short internodes, and the leaves were mostly alternate rather than opposite—as is usual in cosmos. On one plant exposed to 5 short photoperiods a flower bud formed about the middle of July but soon reverted to vegetative growth. One of the bracts of the lower cycle which had been formed was unusual in that it had one lobe which was pink, like the petals of this variety (fig. 3). The new stems not only had short internodes but were also highly branched, and the leaves were unusually small and finely divided. About August 7 two small flower buds, both lacking the usual lower cycle of bracts, formed on these stems. Their development was unusually slow, but on August 26 one finally opened into a small but symmetrical flower head. The other bud had not opened by the end of the experiment. Flower buds did not form on any of the rejuvenated branches of the other plants.

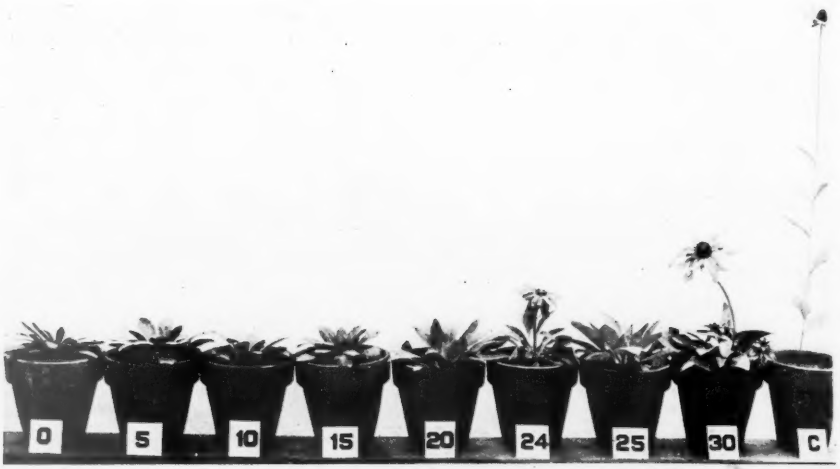
The general order of appearance of the various anomalies was as follows: bract elongation, internodal elongation, incision of bracts, defective flower heads, abor-

tive buds, and reversion to vegetative growth. These usually appeared earliest in the plants exposed to the fewer numbers of short photoperiods and appeared in increasing abundance and extremity of modification with time.

COSMOS SULPHUREUS.—This experiment extended from June 17 to August 24. Blooms first appeared on August 10, on plants exposed to 8, 9, 11, and 12 short photoperiods; and by August 14 even plants exposed to as few as 3 short photoperiods were blooming. Plants exposed to 3 or more short photoperiods continued to bloom in considerable abundance, but not so extensively as those retained under short photoperiods, which began blooming August 1 and bloomed profusely until the end of the experiment. Plants exposed to 2 short photoperiods first bloomed August 21, and on August 16 a single bloom appeared on a plant exposed to one short day. On August 20 and again on August 23 a plant retained under long photoperiods bloomed. None of the plants had anomalous bracts or floral modifications. The effect of the short photoperiods on the height of the plants was similar to that in *C. bipinnatus*.

RUDBECKIA HIRTA.—On July 20, 63 days after planting, all plants exposed to 29 or fewer long photoperiods were still rosettes, although those exposed to 9 or more long photoperiods had somewhat larger leaves than the others. The plants exposed to 30 long photoperiods were beginning to form peduncles, and those retained under long photoperiods were developing elongated stems. Flower buds were forming on plants of both these groups. On August 9 one plant exposed to 30 long photoperiods and one retained continuously under long photoperiods bloomed. By August 17 all plants retained under long photoperiods and two exposed to 30 long photoperiods were blooming, and a small flower head had formed on one plant exposed to 24 long photoperiods. By August 26 all plants exposed to 30 long photoperiods, two exposed to 27, and one each exposed to 23, 24, 26, 28, and 29, were blooming. A "vegetative flower" had developed on a plant exposed to 17 long photoperiods, a number of these also being present on other plants which had the usual type of flower heads as well. By the end of the experiment on November 4, plants exposed to 17, 23, 24, and 26 or more long photoperiods had bloomed, although the plant exposed to 17 long photoperiods bore only the single vegetative flower previously mentioned.

Only the plants retained continuously under long photoperiods developed the usual tall stems present in blooming plants of this species; they averaged 43.5 cm. in height. The other plants remained in the rosette condition (fig. 4). Those which bloomed usually had short peduncles, but one plant each in the groups exposed to 23 and 29 long photoperiods had sessile flower heads. The peduncles ranged in height from 2.0 to 16.0 cm., averaging 6.7 cm. Although the 16-cm. peduncle was developed by a plant exposed to 30 long photoperiods, there was no definite correlation between the number of long photoperiods and the lengths of the peduncles.



FIGS. 4, 5.—Figures indicate number of long photoperiods to which plants were exposed. Fig. 4 (above), representative plants of *Rudbeckia hirta*, September 1. *C*, plant retained under long photoperiods after 28 days from planting. Leaves of plants exposed to long photoperiods are elevated more than those of plant retained under short photoperiods. Two small "vegetative flowers" visible (below the large flower head, formed earlier) on plant exposed to 30 long days. Fig. 5 (below), representative groups of *Matricaria parthenoides* plants, August 1, showing greater size of those exposed to greater numbers of long photoperiods, and dead control plants retained continuously under short photoperiods.

The plants which failed to bloom also lacked macroscopic flower buds, but some had elevated rosette leaves, described as evidence of photoperiodic induction by MURNEEK (8).

MATRICARIA PARTHENOIDES.—Short photoperiods had a definite inhibiting effect on the vegetative development of plants of this species. By July 20 the set of plants retained continuously under short photoperiods had grown very little. There was a general increase in size of the plants exposed to long photoperiods with an increase in the number of these induction photoperiods. These inequalities gradually became less apparent, and eventually some of the plants exposed to intermediate numbers of long photoperiods developed leaves as large as those on plants exposed to the greater numbers. The plants retained continuously under short photoperiods failed to grow beyond a certain stage, however, and between July 20 and 30 all four plants of this group died (fig. 5). Their maximum height was 1.5 cm., measuring from the soil line to the tips of the leaves, while at the same time plants exposed to long photoperiods ranged from 5 to 20 cm. in height. Even the plants exposed to only one long photoperiod thrived.

By September 1 all the plants retained continuously under long photoperiods were blooming, and all had elongated stems averaging 31 cm. in height. All the other plants were still rosettes, and there was no evidence of flower buds on any of them. During September, plants in the groups exposed to 10 and 15 long photoperiods bloomed, and one plant exposed to 13 long photoperiods finally bloomed on November 2. None of the other plants bloomed, and none had flower buds or elongated stems. Blooming never occurred in this species without previous stem elongation, and none of the flowers was unusual in structure.

CENTAUREA CYANUS.—Between July 20 and 24 the plants retained continuously under long photoperiods and one plant exposed to 20 long photoperiods were exhibiting stem elongation, and by July 31 flower buds were present on all of them. On August 10 a flower bud appeared on a plant exposed to 17 long photoperiods, but this never opened. The flower head on the plant exposed to 20 long photoperiods was small and unsymmetrical. The plants retained under long photoperiods began blooming August 15 and continued to bloom in the usual manner. At this time one plant each in the groups exposed to 5, 7, and 10 long photoperiods, and most of the plants exposed to 12 or more, had elongated stems 6–16 cm. in height. These stems were unusually thick and fleshy. The plants retained continuously under long photoperiods had stems averaging 36.5 cm. in height. By the close of the experiment on October 3, plants exposed to 10, 12, 13, and 15 long photoperiods had bloomed. All plants which bloomed had elongated stems.

COREOPSIS TINCTORIA.—During the last week in July stem elongation began in the plants retained under long photoperiods, and buds began appearing on these plants August 2. By this date stem elongation was also occurring on a single

plant of the groups exposed to 18, 19, 23, 25, 28, and 30 long photoperiods. The plants retained under long photoperiods began blooming August 17 and continued to bloom profusely until the end of the experiment on September 3. One flower bud opened on August 24 and two on August 26 on a plant exposed to 18 long photoperiods, but no further anthesis occurred on this plant. Before the termination of the experiment, plants exposed to 23, 24, and 27 or more long photoperiods had also bloomed sparsely. Stem elongation was evident in one plant exposed to 4 long photoperiods, two exposed to 9, one exposed to 18, and in one or two plants of each group exposed to 20 or more long photoperiods. All plants retained under long photoperiods had elongated stems averaging 85 cm. in height, in comparison with 40 cm. for plants returned to short photoperiods. Only plants with elongated stems bloomed, and no unusual floral structures developed.

## Discussion

After a plant has initiated flower primordia or a particular type of vegetative development under the influence of certain photoperiods (photoperiodic induction) and then is transferred to photoperiods not favorable for the initiation of such development, one of two things—or intergradations between them—may occur. The development may continue as a photoperiodic after-effect, or it may be inhibited (photoperiodic inhibition). When flower primordia are thus inhibited the plant may become rejuvenated; that is, vegetative development may be resumed. These phenomena should not be confused with what may be termed "obligatory photoperiodic change," that is, the fact that in some species the photoperiods favoring the initiation of flower primordia are different in length from those necessary for the continuation of reproductive development (**5**), or with "photoperiodic vernalization" (**10**), in which photoperiods of a certain length favor the initiation and completion of reproduction but must be preceded by photoperiods of a different length or some other vernalizing agent which favors production of "ripeness to flower."

In the present study photoperiodic after-effects were evident in all six species, as indicated by the formation of flower buds, flower heads, and in some species fruits, following transfer of the plants to photoperiods not favoring the initiation of flower primordia from photoperiods favoring such initiation. These after-effects were much less extensive and occurred only after exposure to a greater number of induction photoperiods in the long-day than in the short-day species. This may eventually be found to be a more or less general difference between long- and short-day species. Although HAMNER and NAYLOR (**4**) observed after-effects in dill plants exposed to only a few long photoperiods, MURNEEK (**8**) later pointed out that dill will eventually reproduce under short photoperiods and should conse-

quently be considered a neutral or intermediate rather than a true long-day species.

Short photoperiods retarded the vegetative growth of both the long- and short-day species studied. This was most extreme in *Matricaria*, marked stunting and subsequent death occurring in the plants retained continuously under short photoperiods, while plants exposed to even one long photoperiod thrived, although they did not become so large as those exposed to more long photoperiods. There was no clear case of the persistence of short-day inhibition of vegetative growth as an after-effect. Although the independence of photoperiodic after-effects as regards flower formation and photoperiodic inhibition of stem elongation reported by MURNEEK (**7, 8**) for *Rudbeckia* was evident in *R. hirta* in this study, it did not occur in any of the other long-day species and obviously could not occur in the short-day *Cosmos* plants. In these other long-day species stem elongation occurred in some of the plants exposed to greater numbers of long induction photoperiods and may be considered a photoperiodic after-effect. Although these stems were not so tall as those of plants retained continuously under long photoperiods, only plants with elongated stems bloomed. Apparently stem elongation and flower-head formation are not independent in all long-day composites.

The vegetative flowers of *Rudbeckia* and the anomalous bracts and flower heads of *Cosmos bipinnatus* may be considered as evidence of partial reversion to vegetative development (rejuvenation), while in the latter species actual resumption of vegetative growth from flower buds occurred in some plants. This is in the same category as the rejuvenation of hemp observed by SCHAFFNER (**13**). The anomalous structures in *C. bipinnatus* represent a graded series of reproductive-vegetative interphases: elongation of bracts, stem elongation between bracts, incision and foliation of bracts, formation of defective flower heads and abortive buds, and finally actual resumption of vegetative growth from the flower bud. Except for the vegetative flowers of *Rudbeckia*, no such interphases occurred in the other species. Their absence in *Cosmos sulphureus* was evidently due to the fact that long photoperiods in conjunction with relatively high temperatures are favorable for reproductive development in the Orange Flare variety used. In view of the scanty after-effects in *Matricaria*, *Coreopsis*, and *Centaurea*, numerous interphases might be expected, but none occurred. This seems to indicate that such interphases are not produced by all species of composites.

Since the anomalous bracts and flower heads in *Cosmos bipinnatus* occurred earliest in the plants exposed to the smallest numbers of short photoperiods and became more pronounced with time, they tend to add weight to CHAILAKHIAN'S (**2**) theory of florigen charge—that during induction a charge of florigen accumulates and is gradually used after transfer to photoperiods not favorable for initia-

tion of primordia. They would be more difficult to explain if we accept the theory (6) that during induction there is a permanent change in metabolic processes which makes possible the continuation of florigen synthesis, even after transfer.

Since the short induction photoperiods greatly increased the abundance and earliness of bloom in the Orange Flare *Cosmos*, apparently it should not be considered a strictly neutral or intermediate plant at high temperatures, even though it can initiate flower primordia under both long and short photoperiods at such temperatures. In this variety short photoperiods are more effective than long ones in initiating flower primordia, even at higher temperatures.

## Summary

1. Photoperiodic after-effects were observed in two short-day species (*Cosmos bipinnatus*, *C. sulphureus*) and four long-day species (*Rudbeckia hirta*, *Matricaria parthenoides*, *Centaurea cyanus*, *Coreopsis tinctoria*). Twenty-eight days after planting the seed, the plants being kept under photoperiods unfavorable for reproductive development, they were subjected to from 1 to 20 or 30 induction photoperiods of a length which favored initiation of flower primordia. They were then transferred to photoperiods not favoring such development and retained there for the duration of the experiments.

2. In *Cosmos bipinnatus* anthesis occurred in some plants exposed to 5–11 short photoperiods, and in abundance in all plants exposed to 12 or more short photoperiods. In the Orange Flare variety of *C. sulphureus* even the plants retained continuously under long photoperiods eventually bloomed sparsely, but the abundance of flowers increased with an increase in the number of short induction photoperiods. Plants of the long-day species *Rudbeckia hirta*, *Matricaria parthenoides*, *Centaurea cyanus*, and *Coreopsis tinctoria* bloomed sparsely after exposure to minima of 17, 10, 10, and 18 long photoperiods, respectively.

3. Photoperiodic inhibition of stem elongation by the short photoperiods of the transfer environment occurred in *R. hirta*, even in the plants which bloomed. In the other three long-day species such inhibition of stem elongation was not universal, and only plants with elongated stems bloomed.

4. The short photoperiods inhibited the vegetative growth of both the long- and short-day species. In *Matricaria* the plants retained continuously under short photoperiods reached a total height of only 1.5 cm. and soon died, while plants exposed to as little as one long photoperiod thrived.

5. Reproductive-vegetative interphases, which may be considered as initial stages in rejuvenation, occurred in two species. A number of "vegetative flowers" with green petals and more or less vegetative stamens and pistils developed on plants of *Rudbeckia hirta*. In *Cosmos bipinnatus* there were many elongated bracts, incised and foliaceous bracts, elongated internodes between bracts, and defective

flower heads and abortive buds. Some plants of this species actually resumed vegetative growth from young flower buds. One plant exposed to 5 short photoperiods made a double reversal in its terminal bud, changing from vegetative to reproductive growth, back to vegetative growth, and once more to reproductive growth.

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## LITERATURE CITED

1. BIDDULPH, O., Histological variations in cosmos in relation to photoperiodism. BOT. GAZ. **97**:139–155. 1935.
2. CHAILAKHIAN, M: K., Concerning the hormonal nature of plant development processes. Compt. Rend. Acad. Sci. U.R.S.S. **16**:227–230. 1937.
3. GARNER, W. W., and ALLARD, H. A., Further studies in photoperiodism: the response of the plant to the relative length of day and night. Jour. Agr. Res. **23**:871–920. 1923.
4. HAMNER, K. C., and NAYLOR, A. W., Photoperiodic responses of dill, a very sensitive long-day plant. BOT. GAZ. **100**:853–861. 1939.
5. LOEHWING, W. F., Photoperiodic aspects of phasic development. Science **90**:552–555. 1939.
6. LUBIMENKO, V. N., and SZEGLOVA, O. A., Sur l'induction photopériodique dans le processus du développement des plantes. Bull. Bot. Gard. Leningrad **1–2**:1–52. 1931.
7. MURNEEK, A. E., A separation of certain types of response of plants to the photoperiod. Proc. Amer. Soc. Hort. Sci. **34**:507–509. 1936 (1937).
8. ———, Length of day and temperature effects in *Rudbeckia*. BOT. GAZ. **102**:269–279. 1940.
9. POESCH, G. H., and LAURIE, A., The use of artificial daylight and reduction of the daylight period for flowering plants in the greenhouse. Ohio Agr. Exp. Sta. Bull. 559. 1935.
10. PURVIS, C. N., and GREGORY, F. G., Studies in the vernalisation of cereals. A comparative study of vernalisation of winter rye by low temperature and short days. Ann. Bot. N.S. **1**:569–591. 1937.
11. ROBERTS, R. H., and STRUCKMEYER, B. E., The effects of temperature and other environmental factors upon the photoperiodic responses of higher plants. Jour. Agr. Res. **56**:633–678. 1938.
12. ———, Photoperiod, temperature and some hereditary responses of plants. Jour. Hered. **29**:95–98. 1938.
13. SCHAFFNER, J. H., The change of opposite to alternate phyllotaxy and repeated rejuvenation in hemp by means of changed photoperiodicity. Ecology **7**:315–325. 1926.

# INTERRELATION OF ORGANIC MATERIALS IN THE GROWTH SUBSTANCE RESPONSE

S. C. BAUSOR

(WITH FOUR FIGURES)

## Introduction

The manner in which growth substances influence cellular activity is one of the more pressing problems in plant physiology. The diverse effects induced by these substances and the low concentrations in which they are effective suggest that their function may be regulatory, but not in a specific way. At least no single effect can be ascribed to them. The response is of a general nature, and normal cellular activities are accelerated or depressed. Mature cells may be rejuvenated to renewed activity, their relation to neighboring cells and tissues may be altered, and they may assume embryonic qualities such as enlargement, increment in protoplasmic content (especially marked in the nucleus), and mitoses. This chain of events is often followed by organization of new structures such as roots or shoots, or merely galls, some of the cells of which may mature as vascular strands. Occasionally mature cells do differentiate, or differentiate differently from normal, as in certain parenchymatous cells of the pith described later which become reticulately thick walled. Apparently the key to the problem of senescence is to be found in the reaction of the growth substances.

## Literature review

The object of the present work was to determine the histological and cytological changes resulting from the interaction of growth-regulating substances and nutrients supplied to cuttings. Many researches have already appeared which shed some light upon the role played by growth-regulating substances in the metabolism of the plant.

There is considerable increase in water content in tissues affected by growth substances. STUART (**29**) reported an increase in fresh weight of bean cuttings accompanied by an increase in pectic compounds, when treated with indoleacetic acid in lanolin, and MITCHELL (**16**) found an increased percentage of water in regions of bean plants treated with lanolin mixtures of naphthaleneacetic acid or the amide.

In respect to dry matter, MITCHELL and HAMNER (**17**) found an increase in dry weight of beans treated with application of high dilutions of indoleacetic acid in lanolin. On the other hand, with high concentrations of growth substance less or-

ganic matter was synthesized than in the controls, according to the researches of ALEXANDER (1) using indoleacetic acid and MITCHELL (16) comparing naphthaleneacetic acid and naphthalene acetamide. MITCHELL, however, discovered that the nitrogen content was appreciably higher in the treated plants than in the controls, but the distribution of the nitrogen was different with respect to the two compounds. The gall formed at the decapitated apex in plants treated with naphthaleneacetic acid contained about a third of the total nitrogen, but in the case of the acetamide, where lignification is a characteristic response (13), only slightly more nitrogen accumulated in the apical region than in the controls.

Another effect of the growth substances is the mobilization of materials toward the regions of vigorous growth and a redistribution of storage materials in the plant. The accumulation of nitrogen in the apical gall just mentioned is a case in point. ALEXANDER (1) found a similar movement of carbohydrates toward the apical swelling and an accumulation of starch below it. MITCHELL and MARTIN (19) indicated that the direction of flow of materials from the cotyledons of etiolated bean plants was determined by indoleacetic acid.

STUART and MARTH (30) observed an accumulation of sugars in the stems of *Ilex opaca* cuttings when treated with indolebutyric acid. BORTHWICK, HAMNER, and PARKER (5) showed by microchemical tests an increase in proteins and a decrease in starch in regions of the tomato stem where cell divisions were initiated by indoleacetic acid. Starch accumulated below this region.

STUART (29) showed the mobilizing effect of indoleacetic acid upon bean cuttings up to the time of root emergence, in which the rate of movement of materials from the leaves to the hypocotyl was greatly accelerated. Dry weight, nitrogen content, and pectin increased in the hypocotyls over the controls. Sugars at first accumulated faster in the hypocotyls of the treated plants but later became less than in the controls. After root emergence, MITCHELL and STUART (20) found that nitrogen decreased in hypocotyls of experimental and control plants, but much more rapidly in the former. They concluded that indoleacetic acid affected the proteolytic enzymatic action.

Mobilization seemed to be effected through the action of the growth substances on the enzymatic system. MITCHELL, KRAUS, and WHITEHEAD (18) showed that naphthaleneacetic acid sprayed upon the leaves of kidney bean plants hastened the hydrolysis of starch and dextrins in the leaf. Sugars accumulated at first but later fell to the level of the controls. Leaves depleted of starch and dextrins by being kept in the dark accumulated less starch, dextrins, and sugars when placed in the light and sprayed than did the controls. MITCHELL and WHITEHEAD (21) extended this investigation, reporting that hydrolysis of starch followed upon spraying bean leaves with indoleacetic, indolepropionic, indolebutyric, naphthaleneacetic, and naphthoxyacetic acids. A lesser effect on starch was obtained with phenyl-

acetic acid, and none at all with naphthalene acetamide. The increase of sugars in holly stems (30) presumably resulted from the hydrolysis of starch in the leaves, since only a trace of starch was present in the stems. BEAL (3) illustrated the disappearance of starch from the endodermis of bean stems treated with indoleacetic acid.

The interrelation of other materials with the growth substances has been reported in several publications. FLIRY (10) demonstrated the resumption of elongation in decapitated sunflower hypocotyls by the application of either indoleacetic acid or sugars (sucrose and glucose) to the cut surface. SWEENEY and THIMANN (31) showed that the transient effect of streaming in the protoplasm of *Avena* epidermis could be continued for 2 hours in a 1 per cent solution of fructose. SCHNEIDER (26) reported increased growth of sections of oat coleoptiles when sucrose, maltose, dextrose, levulose, or endosperm extract was added to the test solution of indoleacetic acid. Mannitol appeared to be without effect. Both sugar and indoleacetic acid in the medium gave better results than either alone.

COOPER (6, 7, 8), working on root responses, especially of *Citrus* cuttings, observed that if the end of the cuttings, treated with an aqueous indoleacetic-acid solution, were removed the effect of the growth substance was voided and further application of the acid did not lead to the formation of more roots than the controls. He concluded that another substance, rhizocaline, besides the growth substance was necessary for root formation. Failure to bring about rooting in apple cuttings was explained as due to deficiency in rhizocaline. WENT (32), in an elaborate series of experiments with etiolated pea seedlings, proposed the interaction of different specific growth substances, the calines, with auxin in such different growth responses as stem and petiole elongation, root formation, and development of the leaf. BONNER, HAAGEN-SMIT, and WENT (4) showed that an aqueous extract obtained by leaching the cotyledons of peas and other plants greatly increased growth of the mesophyll of the leaf. The solids of the diffusate contained more than 50 per cent carbohydrates, but the accelerated growth of the leaf tissues was not a sugar effect, since the tissues were cultured in 1 per cent sucrose. WENT later (33) suggested that light was necessary for the activity of phyllocaline.

A relation has been shown to exist between the growth substances and some of the micro-elements. EATON (9) reported that boron could be replaced by indoleacetic acid in the nutrition of cotton plants and suggested the formation of auxin as one of the functions of boron. SKOOG (27) recorded an unpublished work of HOAGLAND in which indoleacetic acid seemed to improve the stunted growth of zinc-deficient plants. SKOOG found the absence of auxin in terminal buds and stem sections of zinc-deficient plants and a lower auxin content in their leaves. A decrease in auxin content occurred before the symptoms of zinc deficiency were apparent, while an increase in auxin was detected after zinc salts were added to the

nutrient of the deficient plants. He determined that indoleacetic acid sprayed on the leaves increased stem growth only in the early stages of deficiency and concluded that zinc was necessary in the metabolism of the plant to keep the growth substances in an active state and to prevent their oxidation.

## Material and methods

Plants of Bonnie Best tomato and Calapproved kidney bean were used as material. In the experiments employing potted tomato plants, these were about 5 inches high, with three expanded pinnate leaves. Those from which the cuttings were taken were larger, but all cuttings were made 5 inches long and had the three uppermost leaves expanded. Bean cuttings were taken from young plants just above the second node (paired unifoliate leaves). In these the second trifoliate leaf was just expanding.

The mineral solutions used for root cultures by ROBBINS and SCHMIDT (25) and by WHITE (34) were both found in preliminary tests to be satisfactory for keeping the cuttings alive. A modification of WHITE's nutrient medium was used in all experiments reported here, in which potassium iodide, glycine, thiamin, and sucrose were omitted. To this solution a weighed amount of the material under examination was added. The solutions were changed every second day to prevent the growth of molds and the effect of fermentation products.

The cuttings were kept in the dark for 24 hours before being treated with the growth substances and remained in darkness for the duration of the experiment. The growth substances were applied unilaterally to the stems as a 1 per cent lanolin mixture. The temperature of the darkroom was 68°–70° F. The period of treatment with the growth substances in the presence of a special nutrient was 6 days, except when otherwise indicated. At the end of this time fixations were made in Allen and Wilson's modification of Bouin's solution. Controls consisted of hormone-treated cuttings in the mineral solution alone and untreated cuttings in the special nutrients.

Microscopic examination was made of the stem tissues to determine the changes resulting from the interaction of the growth substance and the nutrient supplied to the cuttings. When the reaction was great the typical white swollen areas could be seen macroscopically. For cytological and histological studies, hand sections were stained with iodine in 95 per cent alcohol, counterstained in gentian violet in absolute alcohol, cleared in xylol, and mounted in Canada balsam.

## Experimental results

### TOMATO

Both intact plants and cuttings that remained in darkness during the period of treatment failed to produce the typical proliferations and root initiation which

occur in plants kept in the light. As judged by curvatures, however, stretching growth was not appreciably affected.

ROOTED PLANTS.—Potted tomato plants were treated unilaterally with 1 per cent β-naphthoxyacetic acid and the controls with pure lanolin. To assure results as nearly comparable as possible, the paste was applied on small cardboard strips $\frac{1}{4} \times \frac{1}{8}$ inches, containing approximately equal quantities of the paste. The cards were applied to the middle of the third internode, in line with the uppermost expanded pinnate leaf (the third). With the exception of the curvatures, no apparent differences could be noted between treated and untreated stems after 6 days; but closer examination showed that enlargement of the nuclei took place where the tissues were activated by the growth substance. The large size of the nucleolus was characteristic. In untreated sectors opposite the paste and in comparable regions of untreated plants the nuclei were much smaller and had small nucleoli (figs. 1, 2). This was the sum total of the observable activity induced by the growth substance under these conditions. The same treatment in the light produced the typical white patches indicative of proliferation and root development.

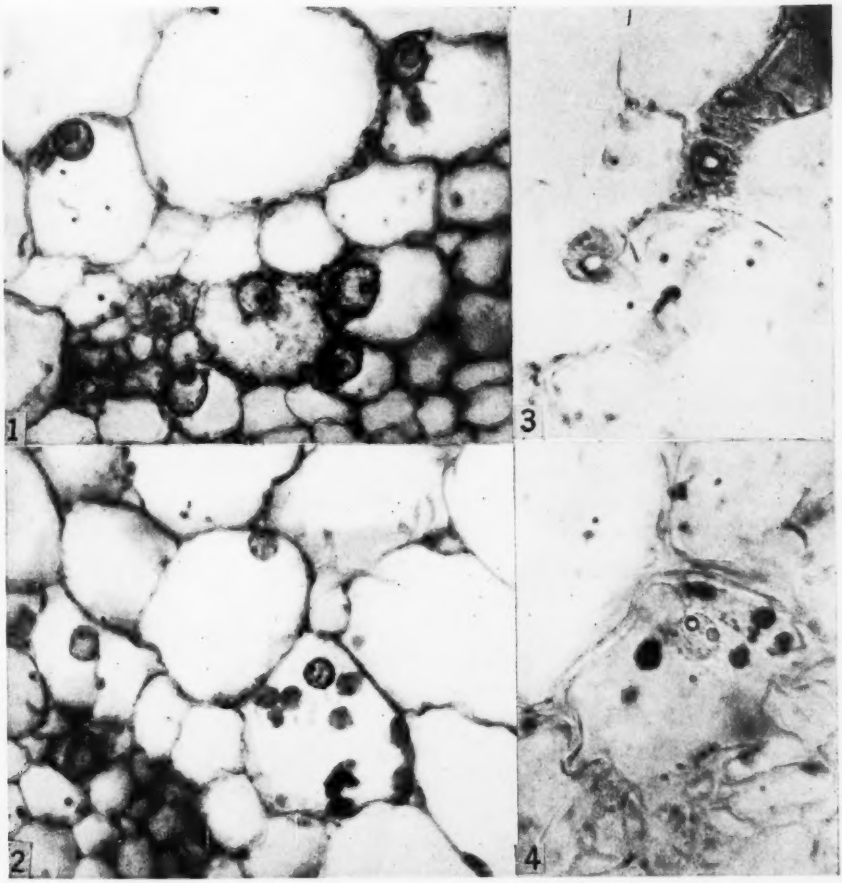
To determine what influence the foliage may have exerted in this reaction, three series of plants treated as just described were set up as follows: (*a*) all leaves removed except the third pinnate one, and plants decapitated; (*b*) all leaves removed, and plants decapitated; and (*c*), as in (*b*), but not decapitated. In series (*a*) the results were similar to those with intact plants, but in (*b*) and (*c*) no nuclear enlargements occurred.

The magnitude of the curvatures in the different series could not be determined accurately, since defoliation in these plants produced curvatures before the growth substance was applied.

CUTTINGS.—In all cuttings the lanolin mixture was applied copiously to one side of the stem by means of a glass rod.

Leafy cuttings maintained in the mineral nutrient showed the same cytological changes as the potted plants. Great enlargement of nuclei and nucleoli occurred (fig. 3) as compared with untreated controls. In the latter, very small nuclei were present, some with small, granule-like, refractive nucleoli, others without distinguishable ones.

In leafless cuttings the nuclei were very small in both experimental plants and controls, and the only apparent differences seemed to be the greater predominance of enucleolate nuclei in the controls. The leafy controls were similar to the treated defoliated cuttings. The growth substances had very little effect upon the metabolism of the tissues under these conditions. The results were the same with indoleacetic acid and β-naphthoxyacetic acid. The effectiveness of the growth substance apparently was dependent upon something diffusing from the leaves.



FIGS. 1–4.—Figs. 1, 2, cross-sections of stems of intact tomato plants: Fig. 1, treated with 1 per cent $\beta$-naphthoxyacetic acid in lanolin; enlargement of nuclei and nucleoli in cells of endodermis and phloem parenchyma. Fig. 2, control, treated with pure lanolin; small nuclei and nucleoli. Fig. 3, section of stem of tomato cutting in mineral nutrient, treated 6 days in darkness with 1 per cent $\beta$-naphthoxyacetic acid in lanolin; enlargement of nuclei and nucleoli. Fig. 4, tomato cutting treated in darkness 6 days with 1 per cent indoleacetic acid in lanolin; enlarged binucleolate nucleus, showing two types of nucleoli.

To investigate the limitation of the growth response in the dark, and its further limitation in defoliated plants, similar cuttings were cultured in the mineral nutrient to which 1 per cent sucrose was added and treated with 1 per cent indoleacetic acid in lanolin. The response of both leafy and defoliated cuttings followed the sequence occurring in plants in the light. Large white macroscopic areas were conspicuous. Typical proliferations occurred in endodermis, pericycle and phloem parenchyma, and cambium, and numerous root primordia developed. Enlargement and division of cells occurred in cortex and pith. Large nucleoli typical of very active cells prevailed. The activity in defoliated cuttings was somewhat less than in leafy ones, which may have been due to the decreased transpiring surface.

The controls in sucrose showed no unusual development, and no differences could be detected between those with leaves and those without. No proliferations occurred, and nucleoli were small in the cells of endodermis, pericycle, and phloem. Evidently the carbohydrate was the immediate limiting factor in the inhibition of the expected response to the growth substance. This was shown again to be the case in the following experiment.

Tomato cuttings were placed in media plus and minus sucrose, and kept in darkness for progressively longer periods before treatment with $\beta$-naphthoxyacetic acid in lanolin. Each group was subdivided into six and treated after 1, 2, 3, 4, 5, and 6 days. All series were subjected to the growth substance for 6 days and differed from one another only in the length of time in the dark before treatment.

The response was similar in all plus-sucrose cuttings, whether treated after 24 hours or after 6 days in darkness. Proliferations were abundant, and numerous root primordia developed. Nuclei, and their nucleoli especially, enlarged to a great size.

Cuttings without sucrose responded progressively less vigorously as the time before treatment was lengthened. After the 24-hour period cuttings showed enlargement of many of the nuclei, with accompanying increase in size of nucleoli in the cells of the endodermis, pericycle, phloem, and cambium. Treated after 2 days in the dark, fewer enlarged nuclei were present in pericycle, phloem, and cambium, while only an occasional endodermal nucleus was found enlarged. Those treated after 3 days in darkness had still fewer enlarged nuclei; and, as in the preceding series, these occurred mostly in pericycle and phloem. Plants treated after 4 days in the dark had no enlarged nuclei. The nucleoli, however, were definite though small and appeared as refractive granules. After 5 days the nuclei were still smaller, and with very small nucleoli which were sometimes difficult to distinguish. By the sixth day nucleoli were not visible.

The condition in cuttings treated after the fourth and fifth days in darkness was similar to that in treated and untreated defoliated cuttings, and in untreated

leafy cuttings after 1 day. Apparently the reserves or assimilated materials of the leaves—and probably also of the stem—were made available to the tissues activated by the growth substances, even under conditions of starvation. Carbohydrates were mobilized in amounts sufficient to result in nuclear enlargements and a synthesis of nucleolar substance. This occurred even after 3 days in the dark before treatment, although fewer nuclei were involved. The disappearance of the nucleolus was delayed in the activated cells until after the cuttings were in darkness for 11 or 12 days. This is almost double the time in which all nucleolar material was used up in untreated or defoliated cuttings.

When carbohydrates were available, one of the first visible effects of the growth substance was the accumulation of proteinaceous reserves in the nucleolus. These were utilized under conditions of inanition.

Enlarged nucleoli were of two types. In some the stainable substance was at the periphery, surrounding a clear central body or vacuole. Occasionally small clear areas were to be found in this peripheral material. In others the stainable material made up the whole body of the nucleolus, and scattered within were numerous (five, six, or more) small clear regions. In binucleolate nuclei both types may be represented (fig. 4).

Curvatures occurred in all the plus-sucrose plants, and in the minus-sucrose plants up to the fifth day in darkness.

An experiment was then devised to determine whether the potentialities of the nuclei were impaired by the presence of a high concentration of growth substance when a limiting supply of carbohydrate was available.

Two sets of tomato cuttings were placed in nutrient solutions, one without a carbohydrate and the other containing 1 per cent sucrose. The plants in the minus-sucrose medium were treated with 1 per cent indoleacetic acid in lanolin, after being in the dark for 24 hours, and remained in this nutrient for 3 additional days. At the end of this time they were transferred to a plus-sucrose medium, and at the same time the plus-sucrose plants, which up to now remained untreated, received an application of the indoleacetic-acid mixture. Five days later treated stems of both series were fixed for study. A concurrent set of cuttings in sucrose treated after 24 hours in darkness was also fixed at this time.

Macroscopic differences in the three sets of cuttings were conspicuous. Both sets cultured from the beginning in media containing sucrose had conspicuous white swellings, which were clearly larger in those treated with the growth substance after 1 day in the dark than in those treated after 4 days. On the other hand, the experimental lot transferred to sucrose after 4 days' treatment showed external evidence of reaction in only two of the ten cuttings. Except for these two, microscopic examination showed less activity in the treated regions than did treated cuttings maintained from the start in the medium containing sucrose. But

the response was not slight in any of the cuttings. Proliferations and root initials were abundant. Thus the typical response was shown, upon the addition of sugar to the medium, by cells containing a relatively high concentration of growth substance and low carbohydrate supply, but to a somewhat less degree than those containing a high carbohydrate supply, derived from the medium, at the time the growth substance was applied.

INTERACTION OF OTHER CARBOHYDRATES.—A comparison of the effectiveness of six sugars in relation to the response to growth substances was made by supplying them to the cuttings as in previous experiments. Three disaccharides and three monosaccharides were used in 1 per cent concentrations: sucrose, maltose, lactose, d-glucose, d-levulose, and d-galactose. All except galactose, which was toxic, were utilized by the cuttings and the typical reaction to the growth substance followed. A semi-quantitative determination of the response was made by comparing the extent of proliferation of endodermis, pericycle, phloem, and cambium; the number of root primordia induced; and the degree of activity in the pith. Three growth substances, indoleacetic acid, β-naphthoxyacetic acid, and ethyl β-naphthoxyacetate, were employed with each of the sugars.

The greatest response was obtained by the use of maltose; levulose and sucrose were only slightly less efficient. Smaller responses were obtained with glucose and lactose in approximately the same degree. Qualitatively the response was similar with all five sugars and conformed to the previous report on Marglobe tomatoes (2). In none of the controls in the different sugars was there any activity. One per cent mannitol was not utilized by the cuttings, judging from the absence of response to β-naphthoxyacetic acid.

RESPONSE IN RELATION TO NITROGENOUS CONSTITUENTS.—Tomato cuttings, cultured in a medium containing sucrose, in which nitrogen was omitted by substituting calcium sulphate for calcium nitrate and potassium nitrate, responded to treatment with the growth substances in no different manner from those in the complete nutrient. Both exhibited the full effects of the growth substance in the presence of a carbohydrate supply. Apparently there was sufficient nitrogen in the cuttings that was easily translocated to the activated tissue; and it leaves open to question the actual utilization of inorganic nitrogen in a nutrient containing it.

NITROGEN-DEFICIENT PLANTS.—Eight tomato plants grown in a minus-nitrogen mineral solution for 1 month, and eight grown in plus nitrogen, were available. The minus-nitrogen plants were very hard. They were 4 inches tall with three small yellow-green leaves. The plus-nitrogen plants were 2.5 feet high, dark green, and rather succulent. Treatments were made on the intact plants in the greenhouse, two of each series with indoleacetic acid and two with β-naphthoxyacetic acid in lanolin. Four served as controls, two lanolin treated and two untreated.

Both plus- and minus-nitrogen plants curved negatively in response to indoleacetic acid and positively to β-naphthoxyacetic acid. Portions were fixed 6 days after treatment.

The nitrogen-deficient plants responded with vigor. Proliferations occurred in the cortex, endodermis, pericycle, and phloem. Root primordia were initiated and several large roots emerged. Nuclei and nucleoli increased to a great size in all activated tissues. The high-nitrogen plants responded only slightly more vigorously. More root initials were formed and larger roots were more abundant. The controls showed no activity, nuclei were smaller than in those treated with the growth substance, and cell division was not apparent.

Eight spindly, nitrogen-deficient tomato plants, about 2 feet high and with light green foliage, were also available. They had grown in a nitrogen-free medium for 8 weeks. Cuttings 5 inches long were taken from their tops. These were placed in a nutrient medium containing 1 per cent sucrose, four plus nitrogen and four minus nitrogen; and after 24 hours in the dark were treated with indoleacetic acid in lanolin. Both reacted similarly, which again questions the utilization of the nitrates by the cuttings. Responses like those of the nitrogen-deficient plants in the light resulted from the hormone treatment.

UREA AND GLYCINE.—One per cent urea or glycine added to the nutrient solution was toxic to tomato cuttings. These were tried in order to determine whether organic nitrogenous compounds could be substituted as respiratory and structural materials in place of the carbohydrates. Treated portions of some of the plants in the nutrient containing urea were fixed 3 days after treating them with indoleacetic acid, in which time the leaves and apex had wilted but the stems to which the paste was applied appeared normal. Sections of these showed that no local activation had occurred.

## KIDNEY BEAN

For comparative purposes the methods used for the tomato cuttings were repeated with the beans, with similar results. The growth response in the dark without an external source of carbohydrate was greatly increased upon the addition of sucrose to the medium. The growth substance was applied unilaterally to the lower part of the third internode.

Without added sugar, nuclear enlargement with accompanying increase in size of nucleolus took place in tissues beneath the paste. In the lower portion of the treated internode (near the node) the endodermis enlarged and divided a few times, and in some cuttings early stages in root initiation occurred at the rays. Any differences between intact and defoliated cuttings were not obvious. The lower portion of the internode gave a greater response than the rest of the internode, even in defoliated cuttings left in darkness 48 hours before treatment, which indi-

cated a higher local supply of sugars in this region of the stem. The amount of activity in intact and defoliated cuttings was undoubtedly due to their large size and to the abundance of storage starch in the stem.

When sucrose was added to the nutrient, great proliferation occurred throughout the treated area and large primordia developed. Galactose was also toxic to bean cuttings and showed the same symptoms as in the tomato.

The response to the growth substances in cuttings of etiolated bean seedlings, maintained in various organic media, was studied. The results will be deferred to a later report. Galactose and glycine, which were toxic to the green cuttings, were not injurious to the etiolated plants. The leaves and apex died in cuttings cultured in the nutrient containing 1.0 per cent urea, but 0.1 and 0.01 per cent concentrations were not toxic.

## Discussion

The interrelation between the carbohydrates and the growth substances, as indicated in this work, shows how closely the two are related in the anabolic metabolism of the cell. In the presence of sugars alone mature cells remained in equilibrium and utilized the sugars apparently for respiratory purposes alone. With a quantity of growth substance present at the same time, the carbohydrates were assimilated and protein synthesis occurred. Under the conditions of the experiments, proteins apparently were resynthesized locally from materials present in other parts of the cuttings not directly affected by the growth substances. The disappearance of stainable nuclear material and the nonutilization of supplied nitrates bears this out. Conversely, considerable material accumulated in the nucleus—and especially in the nucleolus—of cells stimulated by the growth substance.

The high starch content of nitrogen-deficient plants may be due to a deficiency of natural growth substances. This is mere speculation, based upon the strong response of such plants, by both curvatures and histological changes; upon the unusual amount of growth occurring in nitrogen-deficient plants when placed in total darkness (**22**); and upon the effect of growth substances on the hydrolysis of starch. In nitrogen-deficient plants nitrogen does not appear to be the factor limiting growth directly, but lack of vigor may be attributable to the inability of the plant to utilize carbohydrates properly.

Nitrogen is reutilized in the plant (**22, 23**) and is readily available to regions activated by the growth substance. It also seems to be needed in relatively small amounts for growth in locally activated cells. For these reasons it is not likely to be a limiting factor in the rooting response of cuttings, or in other phenomena where high metabolic activity is taking place locally. Since normal development of the higher plants is a succession of local growth phenomena, the ease with which

nitrogen is reutilized must be a stabilizing factor. On the other hand, carbohydrates are apparently needed in larger quantities, and the extent of activity is determined by their availability. In this connection, SMITH, NASH, and DAVIS reported (**28**) that low nitrogen–high carbohydrate bean seedlings treated with indoleacetic acid rooted faster than high nitrogen–low carbohydrate plants. They concluded that "New root primordia were largely influenced by the carbohydrate reserves in the plant."

It was shown that removal of the foliage of intact tomato plants and cuttings completely inhibited the small response in these plants to the growth substances in the dark and that the presence of sugars brought about a complete response. A similar case of a weak reaction (gall production) or none at all has been reported (**14**) in tomato and *Bryophyllum* plants that were defoliated and decapitated, when inoculated with an attenuated strain of the crown-gall organism. Leafy plants produced larger galls in all cases. This was attributed to the diffusion of a growth substance from the leaf, in spite of the fact that 3 per cent indoleacetic acid in lanolin added to the inoculated tissues only slightly increased the amount of proliferation. It seems likely that the difference in vigor between intact and defoliated plants and the difference in the accessibility of carbohydrates are the probable explanations of these results.

The toxicity of galactose is a puzzling problem in plant nutrition. It has been investigated carefully by KNUDSON (**11, 12**) and others and reviewed by MILLER (**15**).

It seems highly probable that more vigorous root induction and better subsequent growth of young roots may be had by pretreating cuttings with a solution of an inexpensive sugar such as sucrose or glucose before using a growth substance. OLIVER (**24**) found that honey stimulated rooting of *Chrysanthemum* and *Thuja*. Any treatment tending to convert stored polysaccharides into sugars may have the same effect. It follows that cuttings should have the maximum number of leaves compatible with retention of water. The available carbohydrate supply may partially explain seasonal variations in the rooting of cuttings.

Further research may show the way to increased crop yields through more efficient utilization of nitrogenous compounds in particular, and also of the carbohydrates, within the plant.

## Summary

1. Intact tomato seedlings and cuttings in a mineral nutrient, kept in darkness during treatment, produced only a small response to the growth substances in 6 days. This was limited to slight enlargement of the cells and an increase in size of nuclei and nucleoli.

2. The reaction was almost completely inhibited when the leaves were removed.

3. Intact and defoliated cuttings cultured in sucrose in darkness produced the complete response obtained in plants in the light. Proliferations and root primordia were formed in abundance. Similar results were obtained with maltose, levulose, glucose, and lactose; mannitol was not effective.

4. Cuttings were left in the dark for 1–6 days before treatment. Those maintained on a sucrose nutrient showed no differences due to pretreatment, but those in the mineral nutrient alone showed progressively less response through the third day and none at all beyond that.

5. Cuttings treated with lanolin paste for 3 days while in the mineral solution were then transferred to the sucrose medium. Six days later, when harvested, the treated regions had proliferated and produced root primordia. The response was not so great, however, as in cuttings cultured in sucrose but not treated until the time the minus-sucrose plants were put into the sugar medium.

6. Bean cuttings in a mineral nutrient in the dark also showed decreased response to the growth substance and complete response when cultured in sucrose.

7. Galactose was toxic to both tomato and bean cuttings.

8. Tomato cuttings in a minus-nitrogen medium responded as vigorously to the growth substances as did those with nitrogen. Nitrogen-deficient plants and cuttings also proliferated and gave rise to root primordia.

9. The most obvious cytological change in response to the growth substances in both the bean and tomato was the great increase in size of the nucleolus. Under conditions of carbohydrate deficiency the nucleolus diminished in size and finally disappeared. No changes occurred in the nucleoli of plants cultured in a sugar medium in the absence of growth substances.

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## LITERATURE CITED

1. ALEXANDER, T. R., Carbohydrates of bean plants after treatment with indole-3-acetic acid. Plant Physiol. **13**:845–858. 1938.
2. BAUSOR, S. C., REINHART, W. L., and TICE, G. A., Histological changes in tomato stems incident to treatment with β-naphthoxyacetic acid. Amer. Jour. Bot. **27**:769–779. 1940.
3. BEAL, J. M., Effect of indoleacetic acid on thin sections and detached segments of the second internode of the bean. BOT. GAZ. **102**:366–372. 1940.
4. BONNER, D. M., HAAGEN-SMIT, A. J., and WENT, F. W., Leaf growth hormones. I. A bioassay and source for leaf growth factors. BOT. GAZ. **101**:128–144. 1939.
5. BORTHWICK, H. A., HAMNER, K. C., and PARKER, M. W., Histological and microchemical studies of the reactions of tomato plants to indoleacetic acid. BOT. GAZ. **98**:491–519. 1937.

6. Cooper, W. C., Hormones in relation to root formation on stem cuttings. Plant Physiol. **10**:789–794. 1935.
7. ———, Transport of root-forming hormone in woody cuttings. Plant Physiol. **11**:779–793. 1936.
8. ———, Hormones and root formation. Bot. Gaz. **99**:599–614. 1938.
9. Eaton, F. M., Interrelations in the effects of boron and indoleacetic acid on plant growth. Bot. Gaz. **101**:700–705. 1940.
10. Fliry, M., Zur Wirkung der Endknospe auf die Hypokotylstreckung des Dikotylenkeimlings. Jahrb. wiss. Bot. **77**:150–184. 1933.
11. Knudson, L., Toxicity of galactose for certain of the higher plants. Ann. Missouri Bot. Gard. **2**:659–666. 1915.
12. ———, The toxicity of galactose and mannose for green plants and the antagonistic action of other sugars toward these. Amer. Jour. Bot. **4**:430–437. 1917.
13. Kraus, E. J., and Mitchell, J. W., Histological and physiological responses of bean plants to alpha naphthalene acetamide. Bot. Gaz. **101**:204–225. 1939.
14. Locke, S. B., Riker, A. J., and Duggar, B. M., Growth substances and development of crown gall. Jour. Agr. Res. **57**:21–39. 1938.
15. Miller, E. C., Plant physiology. New York. 1938.
16. Mitchell, J. W., Effect of naphthalene acetic acid and naphthalene acetamide on nitrogenous and carbohydrate constituents of bean plants. Bot. Gaz. **101**:688–699. 1940.
17. Mitchell, J. W., and Hamner, C. L., Stimulating effect of beta(3)indoleacetic acid on synthesis of solid matter by bean plants. Bot. Gaz. **99**:569–583. 1938.
18. Mitchell, J. W., Kraus, E. J., and Whitehead, Muriel R., Starch hydrolysis in bean leaves following spraying with alpha naphthalene acetic acid emulsion. Bot. Gaz. **102**:97–104. 1940.
19. Mitchell, J. W., and Martin, W. E., Effect of indoleacetic acid on growth and chemical composition of etiolated bean plants. Bot. Gaz. **99**:171–183. 1937.
20. Mitchell, J. W., and Stuart, N. W., Growth and metabolism of bean cuttings subsequent to rooting with indoleacetic acid. Bot. Gaz. **100**:627–650. 1939.
21. Mitchell, J. W., and Whitehead, Muriel R., Starch hydrolysis in bean leaves as affected by application of growth-regulating substances. Bot. Gaz. **102**:393–399. 1940.
22. Nightingale, G. T., The chemical composition of plants in relation to photoperiodic changes. Univ. Wisconsin Agr. Exp. Sta. Bull. 74. 1927.
23. Nightingale, G. T., Schermerhorn, L. G., and Robbins, W. R., The growth status of the tomato as correlated with organic nitrogen and carbohydrates in roots, stems, and leaves. New Jersey Agr. Exp. Sta. Bull. 461. 1928.
24. Oliver, R. W., Honey as a stimulant to the rooting of cuttings. Sci. Agr. (Ottawa) **19**:586–588. 1939.
25. Robbins, W. J., and Schmidt, M. B., Growth of excised tomato roots in a synthetic solution. Bull. Torr. Bot. Club **66**:193–200. 1939.
26. Schneider, C. L., The interdependence of auxin and sugar for growth. Amer. Jour. Bot. **25**:258–270. 1938.
27. Skoog, F., Relationship between zinc and auxin in the growth of higher plants. Amer. Jour. Bot. **27**:939–951. 1940.
28. Smith, O., Nash, L. B., and Davis, G. E., Chemical and histological responses of bean plants grown at different levels of nutrition to indoleacetic acid. Bot. Gaz. **102**:206–216. 1940.

29. STUART, N. W., Nitrogen and carbohydrate metabolism of kidney bean cuttings as affected by treatment with indoleacetic acid. BOT. GAZ. **100**:298–311. 1938.
30. STUART, N. W., and MARTH, P. C., Composition and rooting of American holly cuttings as affected by treatment with indolebutyric acid. Proc. Amer. Soc. Hort. Sci. **35**:839–844. 1937.
31. SWEENEY, B. M., and THIMANN, K. V., Effect of auxins on protoplasmic streaming. II. Jour. Gen. Physiol. **21**:439–461. 1938.
32. Went, F. W., Specific factors other than auxin affecting growth and root formation. Plant Physiol. **13**:55–80. 1938.
33. ———, Effects of light on stem and leaf growth. Amer. Jour. Bot. **28**:83–95. 1941.
34. WHITE, P. R., Glycine in the nutrition of excised tomato roots. Plant Physiol. **14**:527–538. 1939.

# EXTRACTION OF AUXIN FROM MAIZE, FROM SMUT TUMORS OF MAIZE, AND FROM USTILAGO ZEAE[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 541

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## Introduction

This investigation was undertaken to test the hypothesis that if growth-affecting or growth-effecting substances play an important role in the normal growth of plants, then the growth disturbances observed in pathological conditions, such as in the corn-smut tumors being studied, should be correlated with disturbed auxone relations in the infected plants. Although many substances are classified as auxones, only those (the auxins) which can be detected by the standard *Avena* coleoptile technique have been studied here. It is not assumed, however, that auxins thus determined are the sole or primary causes of the responses incited by the pathogen, *Ustilago zeae*, but rather that they may be among the compounds correlated with the abnormal activity of the diseased tissues.

Early in this investigation it became apparent that the current procedures for the extraction of auxin from plant material were not adequate, for various reasons. Work done in this laboratory and elsewhere has shown that quantitative auxin extraction from green tissues is not easily accomplished (**5, 10, 12, 20**). As yet no adequate technique has been found that will remove all the auxin from tissues rapidly and with one extraction. Recently LINK, EGGERS, and MOULTON (**10**) described methods for the preparation of large, thoroughly homogeneous, desiccated samples from which sub-samples can be taken. Material thus prepared is highly suitable for auxin-extraction studies.

In this work an extensive study of the extraction and behavior of auxin from smut tumors of corn was undertaken. Also, for source material, corn stems, corn leaves, corn tassels, and four strains of the corn-smut fungus, *U. zeae*, were used to some extent. This investigation was continued over a period of 3 years, during the course of which about 3000 pans of *Avena* were used in the tests. Most of the experiments have been repeated, some of them many times.

## Material and methods

A strain of Northwestern Dent corn and four strains of *Ustilago zeae* were obtained from Dr. E. C. Stakman of the University of Minnesota. The corn was grown in the greenhouse in garden soil, using 12-inch pots with four plants to each

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pot. The tumors were produced by inoculating corn plants when they were about 1 foot tall. Injections of *U. zeae* were made into the region of the growing point with a hypodermic syringe. Monosporidial cultures of *U. zeae*, designated $10I_1$, $10K_2$, $10J_3$, and $10J_4$, were grown on a liquid 0.1 per cent bacto-tryptone-dextrose medium. To incite tumor production, except for certain solopathogenic strains of the microörganism, it is necessary to mix + and − strains at the time of inoculation. Strains $10I_1$ and $10K_2$ yield only local lesions but no tumors, when injected into the plants separately; when mixed in liquid culture just before inoculation, the two together are highly pathogenic and large tumors result, located principally on the stems and leaves. These two strains were used exclusively to incite the tumors for extraction. Strains $10J_3$ and $10J_4$, being solopathogenic, are each capable, when injected into the growing point, of inciting tumor formation. They were not, however, as virulent as a mixture of + and − strains. Studies were made on the yields of auxins from the mats of the fungus grown on liquid media, and in this work all four strains were tested. About 3–4 weeks after inoculation, tumors appeared and continued to be produced over a period of 2 weeks. In the autumn of 1940, samples of normal leaves and of tumors from stems and leaves were collected, frozen immediately by crushed dry-ice ($CO_2$) mixed with the methyl ester of ethylene (cellosolve). This gives a temperature of −80° C. and freezes the material rapidly. While frozen, the tissues were dried *in vacuo*. The dried material was ground in a Wiley micro-mill to pass a 60 mesh and then stored in an evacuated desiccator over phosphoric pentoxide. The powders proved to be stable and yielded active extracts. The auxin was assayed by means of the standard *Avena* test (**22**), using an initial decapitation period of 2 hours. Photographs were taken 100 minutes after application of agar blocks.

The *Avena* tests were made in a room especially constructed for the purpose. The temperature was held at 24° C., the relative humidity at 88 per cent, and a continuous supply of fresh air was brought in and circulated by fans. For the test plants a strain of Swedish Victory oats sold by Vaughan's Seed Company was used. This seed gave very satisfactory results. Germination was high, the coleoptiles grew at a uniform rate, the leaves pulled easily, and the sensitivity was high. The coleoptiles were calibrated at each test with standard agar blocks containing indoleacetic acid, the concentrations being 0.015 and 0.030 mg. per liter of agar. (The average curvature obtained with the former concentration was about 12°.)

Three extractants were used for the auxin extractions: an aqueous ethyl ether, dry ethyl ether, and water. The aqueous ether was freshly distilled for each experiment over wet $FeSO_4$ and CaO. Dry ether was obtained by treating it with potassium permanganate, drying with calcium chloride, and distilling over sodium; it was stored over sodium in the dark in a refrigerator. Prepared by either method it was always found to be peroxide free. When ether was used as the extractant

the extracts were evaporated to dryness in a water-bath at 50° C. and taken up in 2 cc. of melted 1.5 per cent agar. Serial agar dilutions were then poured into an 8×10×1.5 mm. mold. After hardening, the agar cast was cut into twelve equal blocks, which were applied to the *Avena* coleoptiles. In each dilution tested, twelve coleoptiles were used.

When water was the extractant, the extract was not taken to dryness. Instead, 1 cc. was pipetted from the sample to be tested and mixed directly with 1 cc. of melted 3 per cent agar. The dilutions and blocks were prepared as before. In all the extractions, care was taken not to overheat the preparations, and when the extracts were removed from a vessel the latter was washed two or three times with ether. Whenever duplicate samples were extracted and assayed together, they each gave the same number of units of auxin, within the limits of the variability of the *Avena* test itself.

The results of the tests have been expressed in arbitrary units for convenience of comparison where different materials and different methods of extraction were used. The arbitrary activity unit is defined as that amount of substance which—when put in 1 cc. of 1.5 per cent agar—causes 1° curvature after 100 minutes in the *Avena* test.

## Experimental results

### Ether extraction of fresh material

Extracts of Zea mays.—During this work, fresh leaf samples were collected from corn of various ages and extracted in the usual way. Leaves from young corn plants were invariably found to be low in auxin. The highest auxin yields were obtained from leaves of plants coming into tassel. The tassels themselves were exceedingly high in auxin.

Extracts of corn-smut tumors.—A 50-gm. sample of fresh tumor material from leaves and stems was collected in July, 1940, and placed in peroxide-free wet ether in the refrigerator at 4° C. for extraction. Care was taken to collect only young and medium tumors, older sporulating ones being avoided. The tumors obtained in the greenhouse were white or yellow and contained very little chlorophyll. The tissues, almost intact, were placed in 50 cc. of extractant per gram of fresh material. After 12 hours, the ether was removed from the sample, and the sample was washed with fresh ether and put back in new ether for a second extraction. The ether taken off was evaporated to dryness and tested by mixing the extract with 2 cc. of melted 1.5 per cent agar. Serial dilutions were made from this and assayed in the *Avena* test. The results of a series of five extractions are given in table 1. It will be observed from the table that in the first extraction the *Avena* curvatures are small and there is a lack of proportionality between the dilutions and the curvatures. This lack of proportionality was always observed in the first

extraction of the fresh tissues of tumors. In later extractions this peculiarity is less evident. It seems likely that in the first extraction there are masking substances present which prevent the expression of the auxin. The nature of these substances is unknown. Previous work (**4, 8, 18, 17, 16, 21**) indicates that substances with masking or inhibitory effects are frequently found.

Table 1 shows that after five successive ether extractions the sample was still yielding auxin, perhaps indicating continued auxin production over a period of time by the tumors. More data bearing on this point are presented later. Extractions of tumors from ears of corn collected in the field gave results in agreement with those just reported.

TABLE 1

ACTIVITY OF ETHER EXTRACTS OF FRESH SMUT TUMORS OF MAIZE EXPRESSED IN AVERAGE CURVATURE PER 12 AVENA COLEOPTILES (DEGREES)

| AMOUNT TESTED (GM.)* | TESTING DATES | | | | |
|---|---|---|---|---|---|
| | 7/12/40 | 7/19/40 | 7/22/40 | 7/25/40 | 8/16/40 |
| 50 | 7.1 | 14.3 | 13.9 | 19.2 | 12.9 |
| 25 | 7.8 | 16.8 | 11.2 | 16.9 | 10.0 |
| 12.5 | 8.7 | 17.3 | 3.6 | 12.6 | 3.7 |
| 6.8 | 9.2 | 15.8 | 1.5 | 6.8 | 0 |
| 3.4 | 6.8 | 8.8 | 0 | 3.1 | ..... |
| 1.7 | 2.8 | 4.9 | ..... | 0 | ..... |

* Number of grams of fresh material extracted represented by its portion of extract in 1 cc. of 1.5 per cent agar.

AUXIN CONTENT OF CORN LEAF SHEATH TUMORS COMPARED WITH NORMAL LEAF SHEATHS.—Fresh material was used and treated as already described. After 4 days of extraction the leaf sheath tumors gave forty-eight units of auxin as compared with three units for the normal sheaths. This relationship in which tumors are always higher in auxin than the normal tissues was found when the experiment was repeated and when repeated extractions of the same materials were made.

## ETHER EXTRACTION OF FROZEN VACUUM-DRIED MATERIAL

AUXIN FROM SMUT TUMORS OF MAIZE: EXPERIMENTS WITH DRY ETHER.—Recently the development of the frozen vacuum-drying method for the treatment and storage of plant material has made it possible to compare different methods for the extraction of auxins. Using one finely ground sample of tumors of corn smut, studies were made of procedures for the extraction of auxin by drawing subsamples, treating them in various ways, and comparing the results of the different treatments.

With the dried material available for extraction, absolutely dry ether was prepared and used in making extractions. Dried material gave no activity when dry ether was the extractant. Independently of the present work, and using oven-dried material, THIMANN and SKOOG (**20**) have reported similar findings and concluded that water is necessary for auxin extraction of dry material. That auxin is soluble in dry ether has been demonstrated (**10**). Wet ether extracts of nodules of kidney bean, when dissolved in dry ether and then taken to dryness and assayed, showed the same activity as comparable wet ether extracts when taken up directly in agar. The conclusion is that water plays a part in the progressive liberation of auxin.

There was the possibility that the dried residue extracted by dry ether contained material which could be converted into auxin, if water were present. This was tested by a series of three extractions with dry ether, A, B, and C. Sample A

TABLE 2

ACTIVITY OF ETHER EXTRACTS OF 8 IDENTICAL 20-MG. SAMPLES OF VACUUM-DRIED SMUT TUMORS FROM MAIZE

| DATES OF TESTS | DAYS IN ETHER | UNITS OF AUXIN | DATES OF TESTS | DAYS IN ETHER | UNITS OF AUXIN |
|---|---|---|---|---|---|
| 1/16/41..... | 6 | 72 | 3/4/41..... | 7 | 96 |
| 1/16/41..... | 6 | 96 | 3/4/41..... | 7 | 96 |
| 1/23/41..... | 7 | 96 | 3/8/41..... | 7 | 144 |
| 1/25/41..... | 2 | 56 | 3/8/41..... | 7 | 80 |

was tested without further treatment, B was treated with 1 cc. of water for 14 hours, and C with 1 cc. of water for 3 days. Extracts of A, B, and C were inactive in the *Avena* test, and it was concluded that dry ether does not remove any substance which can later be activated by the addition of water.

AUXIN FROM SMUT TUMORS OF MAIZE: EXPERIMENTS WITH WET ETHER.—Extractions of eight 20-mg. samples of frozen vacuum-dried material were made at different times with wet ether and are compared in table 2. The data are presented in terms of the arbitrary auxin units, defined earlier in the paper. Twenty cc. of ether was used for each sample and kept in the refrigerator at 4° C. The wet ether used in all the following experiments was prepared by shaking 15 cc. of dry ether, prepared according to the method already described, with 0.25 cc. of water. This amount of water saturated the ether. The procedure eliminated one source of variability in the extractions, since the dry ether used in all the extractions came from the same supply and contained the same amount of water. The ether used was in the ratio of 1000 cc. per gram of powder. The material remained in ether 7 days, except in two instances in which one extraction was for 2 days and the other 6 days. From four of the extracts 96 units were obtained (table 2); one gave

56 units, another 80, another 144, and another 72. When extracted for only 2 days, the auxin yield was 56 units. When the period of extraction in ether was the same, the number of units obtained was remarkably uniform. That time is a factor in the extraction process is shown in the following experiment.

Each of four 30-mg. samples of tumor powder, prepared according to the freezing desiccation method, was placed in 20 cc. of wet ether. Sample 1 was extracted one day; sample 2, two days; sample 3, three days; sample 4, seven days. The experiment was arranged so that the extractions were completed simultaneously, and all were assayed simultaneously. For sample 1 the yield was 44 units; for sample 2 it was 80 units; for sample 3 it was 120 units; and for sample 4 it was 320 units. There is good proportionality between time in wet ether and yield of auxin.

Tests to determine whether the amount of extractant used was a limiting factor showed that different quantities of ether resulted in practically the same number

TABLE 3

AUXIN FROM 20 MG. OF VACUUM-DRIED TUMORS FROM MAIZE SAMPLE EXTRACTED WITH SUCCESSIVE 20-CC. VOLUMES OF ETHER

| DATES OF TESTS | DAYS IN ETHER | UNITS OF AUXIN | DATES OF TESTS | DAYS IN ETHER | UNITS OF AUXIN |
|---|---|---|---|---|---|
| 3/31/41..... | 3 | 48 | 5/18/41..... | 10 | 98 |
| 4/18/41..... | 18 | 384 | 5/29/41..... | 11 | 104 |
| 4/23/41..... | 5 | 112 | 6/7/41..... | 10 | 96 |
| 5/8/41..... | 15 | 176 | | | |
| Total to date..... | | | | 72 | 1018 |

of units of auxin. This corroborates the former observation that auxin is gradually liberated from dried material in the presence of water.

Experiments with repeated extractions of material with wet ether give further support to this observation and are of interest in comparison with the repeated extractions of fresh material discussed earlier. From one series of repeated extractions (table 3) it is evident that the fresh and the dried material on extraction gave similar results. When extracted, both yielded auxin over a long period. Here again the yield of auxin is a function of the extraction time. The fresh material differed greatly from the dry powder, however, in that dry material yielded about 100 times as much auxin as the fresh when the same number of grams of each, on a fresh-weight basis, were used in making the extractions.

In the repeated extractions just described, either dry ether or wet ether was used alone. In another experiment, two 30-mg. samples, A and B, were first extracted with wet ether for 14 days and tested, the residues dried by washing with dry ether, followed by a second extraction in dry ether for 8 days, after which these extracts were tested while the residues were placed in wet ether for a third extrac-

tion. In this third extraction, sample A was in wet ether for 5 days and B for 12 hours. The first extraction with wet ether gave for A and B, 320 and 277 units, respectively; the second extraction with dry ether gave 0 units in each case; the final wet ether extracts were active. From sample A after 5 days, 138 units were obtained, while the yield from B after 12 hours was 40 units. This is further evidence of the part played by water and the importance of the time factor in auxin liberation.

The Soxhlet method of extraction with wet ether was tried with the possibility that auxin in the unbound form might be rapidly removed in this way. For the

TABLE 4

ACTIVITY UNITS OF EXTRACTS OF TWO 20-MG. SAMPLES OF POWDER SOXHLETIZED FOR 18 AND 47 HOURS, RESPECTIVELY. FOLLOWING THE 18-HOUR SOXHLETIZATION THIS SAMPLE WAS EXTRACTED BY STANDING IN SUCCESSIVE VOLUMES OF ETHER IN ICEBOX

| DATE OF EXTRACTION | DAYS IN ETHER | SOXHLETIZED 18 HOURS | SOXHLETIZED 47 HOURS |
|---|---|---|---|
| 3/21/41 | ..... | 288 (standing) | 384 |
| 3/22/41 | 1 | 14 | ..... |
| 3/28/41 | 6 | 28 | ..... |
| 4/12/41 | 16 | 60 | ..... |
| 4/22/41 | 10 | 32 | ..... |
| 5/7/41 | 15 | 28 | ..... |
| 5/29/41 | 21 | 12 | ..... |
| 6/7/41 | 10 | 9 | ..... |
| Total | 79 | 471 | ..... |

tumor material, this does not seem to be the case; almost all the auxin is bound. With short Soxhlet extractions of 3, 6, and 10 hours very low yields were obtained, scarcely greater than if the material had been merely standing in ether for the same periods. Table 4 summarizes the data from two Soxhlet runs, one of 18 hours' duration and the other of 47 hours. It is observed that there is no proportionality between the length of time the material was Soxhletized and the yield of auxin. Other materials extracted in the Soxhlet gave similar results, but no explanation for this behavior is offered.

After the 18-hour Soxhletization, the powder was re-extracted seven times with wet ether, over a period of 79 days. The total auxin yield, including the Soxhletization, was 471 units, as compared with an expected yield of about 1000 units for repeated extractions of the material when left standing in the refrigerator in wet ether. Considerable auxin was lost when the Soxhlet method was used.

Two Soxhlet extractions with dry ether, A and B, were followed by extraction

of A with wet ether and B with water (table 5). There was the possibility that when fats and other substances were removed by dry ether the process of liberation of auxin would be more complete and rapid. After this Soxhletization by dry ether, the material gave similar results in subsequent extractions to powders subjected to repeated wet ether extraction alone, although auxin yields were considerably lower after the Soxhlet procedure.

AUXIN FROM SMUT TUMORS OF MAIZE: WATER EXTRACTION.—A 20-mg. sample of tumor powder was extracted for 2 days in dry ether to which 0.5 cc. of water had been added. By separating the ether fraction from the water fraction and testing each separately for auxin content, it was determined that the water fraction was many times more active than the ether fraction. It seems that the extractable

TABLE 5

ACTIVITY UNITS OF EXTRACTS OF SAMPLES A AND B, EACH SOXHLETIZED WITH DRY ETHER, A THEN EXTRACTED BY STANDING IN WET ETHER 8 DAYS AND B IN WATER 8 DAYS. CONTROLS FOR A AND B EXTRACTED BY STANDING IN WET ETHER AND WATER 8 DAYS IN ICE-BOX, RESPECTIVELY

| A WET ETHER | | B WATER | |
|---|---|---|---|
| SOXHLETIZED, THEN IN ETHER 8 DAYS | CONTROL, IN ETHER 8 DAYS | SOXHLETIZED, THEN IN WATER 8 DAYS | CONTROL, IN WATER 8 DAYS |
| 60 | 192 | 2176 | 2304 |

auxins are highly soluble in water and not very soluble in ether, indicating that the partition coefficient between ether and water is low for these auxins.

These findings suggested some studies on water extraction of the tumor powder. Some of the experiments are similar to those carried out with wet ether. They were devised so that the efficiency of ether and of water as extractants could be compared. Table 6 gives the results of a series of repeated extractions. Ten mg. of powder was put into 2 cc. of water for this series, the ratio of extractant to powder thus being 200 to 1. Experience had shown that more than enough water was present for the amount of powder to be extracted. In making the agar blocks for the *Avena* test, 1 cc. of water extract was put into 1 cc. of 3 per cent agar and dilutions made from this. The powder was put into previously unused distilled water for each subsequent extraction. All extractions were carried out in the refrigerator at about 4° C. Most of the auxin was liberated in the first three extractions. For example, 3008 units (table 8) were obtained from three extractions which

covered 22 days, and only 198 units were obtained in the next three extractions throughout a 33-day period. Comparable repeated extractions by ether and by water were made, and it was found that when water was the extractant the yield of auxin was greater than when ether was the extractant. The total number of units of auxin obtained by exhaustive extraction with water was 3000–3500, while with wet ether only 1000–1500 units were obtained. Greater yields of auxin were obtained in a given time when the material was extracted with water than when ether was the extractant. As an example, in one experiment the auxin yield was

TABLE 6

AUXIN FROM 10 MG. OF VACUUM-DRIED SMUT TUMORS OF MAIZE. SUCCESSIVE WATER EXTRACTIONS EACH OF 2 CC.

| DATES OF TESTS | DAYS IN WATER | UNITS OF AUXIN | DATES OF TESTS | DAYS IN WATER | UNITS OF AUXIN |
|---|---|---|---|---|---|
| 4/21/41 | 9 | 1280 | 5/7/41 | 12 | 104 |
| 4/22/41 | 10 | 1152 | 5/18/41 | 11 | 88 |
| 4/25/41 | 3 | 576 | 5/28/41 | 10 | 6 |
| Total | | | | 55 | 3206 |

TABLE 7

AUXIN FROM 5 MG. OF VACUUM-DRIED SMUT TUMORS OF MAIZE. SUCCESSIVE WATER EXTRACTION EACH OF 2 CC.

| TIME IN WATER | UNITS OF AUXIN | TIME IN WATER | UNITS OF AUXIN |
|---|---|---|---|
| ½ hour | 288 | ½ hour | 0 |
| ½ hour | 32 | 5 days | 128 |

256 units in 2 hours, 512 in 24 hours, and 1536 in 7 days. In sharp contrast is the fact that wet ether gave on the average only 96 units in 7 days.

Another proof that auxin does not all come out at once when water is the extractant was provided by the following experiment. In this case 5 mg. of powder was put into 2 cc. of water for ½ hour. The water was removed from the sample and the latter again put into 2 cc. of water. The material was treated thus with water three times for ½ hour each time. Each 2 cc. of water removed was tested for auxins. Auxin was obtained in the first two extractions (table 7) but not in the third. The powder, apparently exhausted of auxin, was extracted again for 5 days. Tests showed 128 units present at the end of that time. Liberation of auxin must have occurred during the 5-day interval. The experiments were carried out at

2° C. and there was no growth of microörganisms. The experiment was repeated with similar results.

In the experiment (table 7) in which the powder was Soxhletized with dry ether and then extracted 8 days with water, it is noted that 2176 units were recovered, compared with 2304 units from control material not Soxhletized. Thus, removing fats and other substances with dry ether subsequent to water extraction did not aid the process of extraction.

Using water extracts, the effect of heat on the auxin extracted by water from smut tumors was studied. Ten mg. of powder was extracted in 4 cc. of water for 20 hours. At the end of this period, the water was divided into four aliquots. Sample 1 was placed in the icebox at 1.7° C., vials containing samples 2, 3, and 4 were

TABLE 8

EFFECT OF TEMPERATURE ON AUXIN STABILITY; EXPOSURE 2 HOURS IN WATER

| SAMPLE | TEMPERATURE (° C.) | UNITS OF AUXIN | SAMPLE | TEMPERATURE (° C.) | UNITS OF AUXIN |
|---|---|---|---|---|---|
| 1..... | 1.7 | 64 | 3..... | 60 | 72 |
| 2..... | 40 | 64 | 4..... | 100 | 44 |

placed in hot-water baths at 40°, 60°, and 100° C., respectively, for 2 hours. The activity of the several extracts was then tested (table 8). The heat did not change the amount of auxin in the samples under the conditions of the experiment, except at 100° C.

### ETHER EXTRACTION OF FOUR STRAINS OF USTILAGO ZEAE

Four strains of *U. zeae* were grown in a liquid bacto-tryptone medium. After 2 months the mats of the fungus were removed from the surface of the culture medium and placed in wet ether for extraction. The ether was then decanted off, the mats washed several times with fresh ether, and returned to fresh ether for a second extraction. The ether was taken to dryness after each of the two extractions, and the extracts were tested for auxins. It was found that almost all the auxins were removed in the first 24-hour extraction period. When third extractions were made no auxins were obtained. This is in contrast to green tissues which continue to yield auxins, even after ten or more extractions.

For another series of experiments the mats of the fungus were frozen and vacuum dried and the material then ground to a fine powder. The powders were treated with wet ether. Analyses showed the total auxin obtained to be greater than the total obtained by treating comparable fresh mats with ether. Experience has shown that no auxin is obtained when dry ether is used in the extraction of the dry powders.

Strains $10J_3$, $10J_4$, $10I_1$, and $10K_2$ were grown on two different media in order to determine whether or not different amounts of auxin could be obtained from the various strains grown on an organic bacto-tryptone medium, and also whether auxin could be obtained from mats grown on a synthetic medium containing inorganic salts and dextrose but no proteins or amino acids. The four strains were grown for two different periods (2 months and 9 months) on each of the two media. The mats were then frozen and vacuum dried. Ten-mg. samples of the strains of the fungus grown on the organic bacto-tryptone medium were extracted with 20 cc. of wet ether. Eighty-mg. samples of the fungus grown on the synthetic medium were extracted at the same time. Table 9 shows that extracts of the four

TABLE 9

ACTIVITY OF ETHER EXTRACTS FROM FOUR STRAINS OF USTILAGO ZEAE. EACH STRAIN GROWN ON ORGANIC BACTO-TRYPTONE MEDIUM OR ON SYNTHETIC MEDIUM FREE OF PROTEINS AND AMINO ACIDS

| Strain | Bacto-tryptone medium | | Synthetic medium |
|---|---|---|---|
| | Two months on medium, units of auxin from 10-mg. sample | Nine months on medium, units of auxin from 10-mg. sample | Two months on medium, units of auxin from 80-mg. sample |
| $10J_3$ | 42 | 200 | 23 |
| $10J_4$ | 10 | 100 | 32 |
| $10I_1$ | 8 | 160 | 4 |
| $10K_2$ | 4 | 60 | 8 |

strains differ in the amount of auxin which can be obtained by extracting the powders. The extracts from the solopathogenic forms, except in one case, show a higher auxin content than the extracts from the non-solopathogenic ones. It can also be seen that auxin can be obtained from the fungus grown on the synthetic medium free of proteins and amino acids.

The powders from the fungus grown on the organic medium, however, yield about eight times as much auxin as the same amounts of powders from fungus grown on the synthetic medium. In the experiments just described the mats alone were tested for auxins. The next step was to test the liquid media that had supported the fungus.

Forty cc. of both types of liquid medium that had supported the mats of the fungus were dried separately *in vacuo*. The residues were dissolved in 2 cc. of water and mixed with 2 cc. of melted 3 per cent agar. The agar blocks for the *Avena* test were cast from this mixture. From both types of medium very active

extracts were obtained. They were about equally auxinic. The source of the auxin is not known. The organisms may synthesize auxin intracellularly, followed by its diffusion from the cells into the medium, or the cells may produce substances which diffuse into the medium and convert materials in the nutrient to auxin. The fungus on the bacto-tryptone may convert the tryptophane or other organic compounds (either intercellularly or intracellularly) into auxin. In the case of the fungus grown on the synthetic medium in which dextrose was the only organic substance, it seems likely that the auxins were produced intracellularly. The function of the auxins in the metabolism of the fungus, if they have any function, is not known. Perhaps they are katabolic products.

## Discussion

Total auxin yields from frozen vacuum-dried powders from ground tumors of corn smut, or leaves, or the fungus *Ustilago zeae*, were higher than from the same objects in the fresh state. The same findings have been reported for kidney bean roots (10), for legume nodules, and for crown gall of tomato. THIMANN and SKOOG (20) noted that material which had been dried by heat gave a decreased yield of auxin, perhaps due to heating.

The results of this work, and of the investigations just cited, indicate that water plays some role in the liberation of auxin from plant tissues. That its action is hydrolytic has been suggested (10). SKOOG and THIMANN (15) have released bound auxin with proteolytic enzymes. Apparently any free auxin present in the living tissues is destroyed during the freezing and dehydrating process, or is fixed during these processes, since none is removed with dry ether—in which it is soluble.

The auxin from smut tumors is highly soluble in water, much more so than in ether, and comparisons of exhaustive extractions made with the two extractants indicate that the process is more rapidly and completely carried out with water. During the slow ether extraction, perhaps much auxin is destroyed by peroxides and otherwise, explaining the higher total yields from the more rapid water extraction.

All the auxin is obtained from *U. zeae* preparations in a single extraction with wet ether. This is in contrast to green tissues requiring ten or more extractions to obtain the same results.

Strains of *U. zeae* produced auxin when grown either on a bacto-tryptone medium or on a synthetic medium without proteins of amino acids. Auxins have been extracted from microörganisms grown on media containing amino acids or proteins (14, 7, 19, 1, 9, 2, 3). BURKHOLDER (2) demonstrated that *Aerobacter aerogenes* and *Escherichia coli* grown on glycerol-mineral salts-agar, in which the sole

source of nitrogen was either $KNO_3$ or $NH_4CL$, produced auxin. GEORGI and BEGUIN (3) reported the elaboration of growth substances by four species of *Rhizobium* and *Bacillus radiobacter* when the culture medium contained tryptophane. They found, however, that when mannitol was the source of carbon and $KNO_3$ the sole source of nitrogen, no active substances were produced in the medium.

Solopathogenic strains of *U. zeae* were found, with one exception, to produce more auxin than non-solopathogenic strains. This ability of the solopathogenic strains may be correlated with the capacity of the former to encite tumors in corn. LOCKE, RIKER, and DUGGAR (13) reported that for *Bacillus radiobacter* no consistent difference in the production of growth substances was observed between the virulent and the attenuated cultures. GEORGI and BEGUIN tested virulent and attenuated strains of *Rhizobium* and also found no correlation between pathogenicity and auxin yield.

HAAGEN-SMIT, LEECH, and BERGEN (6) have isolated crystalline indoleacetic acid from extracts of corn. This is the first time that this acid has been isolated from higher plants. These workers conclude that since the amount of the isolated acid was many times greater than the auxin amount indicated by the normal extraction process, it represented a large part of the auxin present in bound (precursor) form. Thus it is likely that the auxin extracts of corn contained indoleacetic acid and probably other active substances.

Differences in auxin content exist between smut tumors and similar regions of healthy tissues, the tumors being the more auxinic. LINK has found that in all cases where tumor material has been compared with healthy, the former was the more active. In this connection LINK and EGGERS (11) have recently reported on legume nodules and crown gall of tomato.

The higher activity of the tumors could be due to (*a*) the metabolic activity of the pathogen, or (*b*) the activity of the tumor tissues themselves in response to the injury of the host cells by the parasite. It is clear that auxin may be one of the causes of the pathological condition manifested by tumor production.

During the last 5 years many reports have appeared concerning the role of auxins in the physiology of plants. The only conclusion that can be reached from a review of the literature is that there is no simple relationship of growth-regulating substances to metabolism; apparently they play various roles, both direct and indirect. In general, auxins are found in highest concentrations in regions of the plant that are young and actively growing, that is, regions where the metabolic rate is high. It is possible that the presence of large amounts of auxins in the tumors studied is correlated with the increased and abnormal metabolism of the infected tissues.

**Summary**

1. The extraction of auxin by ether and by water from smut tumors of corn and other materials, frozen and vacuum dried, was studied. For a given weight of smut tumors, water extraction yields the greater amount of auxin. Dry ether extracts are not active. Water is necessary for the liberation of auxin from the tissues. Its action may be hydrolytic.

2. Smut tumors of corn yield auxin slowly with either water or ether extraction. The auxin is almost completely removed from the fungus, *Ustilago zeae*, in one ether extraction.

3. Smut tumors from corn leaves or stems yield more auxin than healthy leaves or stems.

4. Strains of *U. zeae* grown on a synthetic medium containing neither proteins nor amino acids are able to produce auxin.

5. Extracts of both types of medium upon which *U. zeae* had grown for 2 months contained much auxin and practically the same amount in each case.

6. The pathogenicity of strains of *U. zeae* seems to be correlated with their ability to produce auxin in a bacto-tryptone or a synthetic medium.

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## LITERATURE CITED

1. BROWN, N. A., and GARDNER, F. E., Galls produced by plant hormones, including a hormone extracted from *Bacterium tumefaciens*. Phytopath. **26**:708–713. 1926.
2. BURKHOLDER, P. R., Production of growth substance by bacteria in media containing specific organic and inorganic nitrogenous compounds. Amer. Jour. Bot. **26**:422–428. 1939.
3. GEORGI, C. E., and BEGUIN, A. E., Heteroauxin production by efficient and inefficient strains of Rhizobia. Nature **143**:25. 1939.
4. GOODWIN, R. H., Evidence for the presence in certain ether extracts of substances partially masking the activity of auxin. Amer. Jour. Bot. **26**:130–135. 1939.
5. GUSTAFSON, F. G., Some difficulties encountered in the extraction of growth substances from plant tissues. Science **92**:266–267. 1940.
6. HAAGEN-SMIT, A. J., LEECH, W. D., and BERGEN, W. R., Estimation, isolation, and identification of auxins in plant material. Science **93**:624–625. 1941.
7. KÖGL, F., and KOSTERMANS, D. G. F. R., Heteroauxin als Staffwechselprodukt niederer pflanzlicher Organismen. Isolierung aus Hefe. Zeitschr. Physiol. Chemie. **228**:113–121. 1934.
8. LARSEN, PAUL, Über Hemmung des Streckenwachstums durch natürlich vorkommende, Atherlösliche Stoffe. Planta **30**:160–167. 1939.

9. LINK, G. K. K., WILCOX, HAZEL W., and LINK, ADELINE D., Responses of bean and tomato to *Phytomonas tumefaciens*, *P. tumafaciens* extracts, β-indoleacetic acid, and wounding. BOT. GAZ. **98**:816–867. 1937.
10. LINK, G. K. K., EGGERS, VIRGINIA, and MOULTON, J. E., Use of frozen vacuum-dried material in auxin and other chemical analyses of plant organs: its extraction with dry ether. BOT. GAZ. **102**:590–601. 1941.
11. LINK, G. K. K., and EGGERS, VIRGINIA, Hyperauxiny in crown gall of tomato. BOT. GAZ. **103**:87–106. 1941.
12. LINSER, H., Zur Methodik der Wuchstoffbestimmung. II. Die Extraktion von Pflanzenmaterial. Planta **29**:392–408. 1939.
13. LOCKE, S. B., RIKER, A. J., and DUGGAR, B. M., Growth substance and the development of crown gall. Jour. Agr. Res. **57**:21–39. 1938.
14. NIELSEN, N., Untersuchungen über einen neuen wachstrumsregulierenden Stoff: Rhozopin. Jahrb. wiss. Bot. **73**:125–191. 1930.
15. SKOOG, FOLKE, and THIMANN, K. V., Enzymatic liberation of auxin from plant tissues. Science **92**:64. 1940.
16. SNOW, R., An inhibitor of growth extracted from pea leaves. Nature **144**:906. 1939.
17. STEWART, W. S., A plant growth inhibitor and plant growth inhibition. BOT. GAZ. **101**:91–108. 1939.
18. STEWART, W. S., BERGREN, W., and REDEMAN, E. E., A plant growth inhibitor. Science **89**:185. 1939.
19. THIMANN, K. V., On the plant growth hormone produced by *Rhizopus suinus*. Jour. Biol. Chem. **109**:279–291. 1935.
20. THIMANN, K. V., and SKOOG, FOLKE, The extraction of auxin from plant tissues. Amer. Jour. Bot. **27**:951–960. 1940.
21. VOSS, H., Nachweis des inaktiven Wuchsstoffes, eines Wuchsstoff antagonisten und deren Wachstumsregulatorische Bedeutung. Planta **30**:252–285. 1939.
22. WENT, F. W., and THIMANN, K. V., Phytohormones. New York. 1937.

# CARBOHYDRATE NUTRITION OF RHIZOPUS SUINUS

CYRIL J. CORUM

(WITH ONE FIGURE)

## Introduction

Many investigations have been reported concerning the relative suitability of different carbohydrates in supporting the growth of fungi. In no case was attention paid to the purification of the sugars. Several observations on the effect of purification of a sugar upon growth of an organism upon that sugar make this question an important one where nutrition of the fungi is concerned.

Perhaps the earliest work relating to this question was that of WILDIERS (14). In apparently pure sucrose he found a water-soluble substance indispensable for the growth of yeast which could be extracted from the sucrose by 80 per cent alcohol. FUNK and FREEDMAN (5) determined that this substance could be removed from the sucrose by one recrystallization from 80 per cent alcohol. ALLISON and HOOVER (1) reported that one recrystallization of commercial sucrose from hot 70 per cent alcohol removed most of the substance in the sugar which was active as a growth factor for *Rhizobium trifolii*. Extractions of the sucrose in a Soxhlet extractor and evaporation of the extract to dryness gave a product which, although it was 75 per cent sugar, caused increases up to twenty-five fold in the growth of *R. trifolii*. HALL, JAMES, and STEWART (6) reported that recrystallization of sucrose from 80 per cent alcohol resulted in marked reduction in the yeast crop which could be grown by the use of this sugar. The ash obtained from pure sucrose caused no growth stimulation.

Few observations have been made with regard to the effect of the purification of the carbohydrate upon the growth of true fungi. SCHOPFER (11) found that a synthetic medium with purified maltose would not support growth of *Phycomyces* sp., but good growth resulted if Kahlbaum's maltose were substituted. According to SCHOPFER, it must be admitted that there is in the more impure sugar a substance whose role is all important in the development of *Phycomyces*. In the light of present knowledge of the growth relations of this fungus, it is evident that at least one substance present as an impurity in the sugars must have been vitamin $B_1$. STEINBERG (13) refluxed sucrose in a Soxhlet extractor with 95 per cent alcohol for 6 hours, drying the sugar at 103° C. to remove the alcohol. When *Aspergillus niger* was grown in a nutrient solution containing sucrose extracted in this manner, the dry weight production was reduced to 81 per cent of the original amount.

STEINBERG attributes this diminished growth to the removal of zinc and molybdenum. Similar results were obtained when heavy metals were omitted from the culture solution. STEINBERG believes that growth increases obtained with accessory growth materials are due, at least partially, to the presence of heavy metals in these substances.

## Material and methods

Two methods of sugar extraction were used in these experiments. In the first method, 100 gm. of the sugar was stirred vigorously with 500 cc. of absolute alcohol. The mixture was filtered with suction on a Buchner funnel and washed with an additional 500 cc. of alcohol. The sugar was then removed from the funnel, placed in a large beaker, and stirred with 500 cc. of ether. This was also filtered and dried until there was no odor of ether remaining. In the second method, the method just described was preceded by extraction with 500 cc. of acetone, by filtration, and by washing with an additional 500 cc. of acetone. In subsequent discussion, these two methods will be referred to as the alcohol-ether method and the acetone-alcohol-ether method, respectively.

Liquid cultures were employed in these experiments. The medium consisted of various carbon sources used in different concentrations. The mineral nutrient part of the culture solution consisted of:

| | |
|---|---|
| Ammonium nitrate | 0.1M |
| Potassium dihydrogen phosphate | 0.05M |
| Magnesium sulphate | 0.01M |
| Ferric tartrate | trace |

Fifty-cc. portions of the medium were placed in 150-cc. Erlenmeyer flasks and sterilized in an autoclave at 15 pounds' pressure for 20 minutes. Each flask was inoculated with 0.5 cc. of a spore suspension prepared by washing the surface of an agar slant culture with Ringer's solution. Enough of the spore suspension was prepared each time so that an entire series could be inoculated from the same suspension. After 8 days, the mycelium was placed on a previously dried and weighed filter paper, rinsed several times with distilled water, and dried in a hot-air oven at 95° C. for 48 hours. The dried mats were removed from the oven to a desiccator, cooled, and weighed rapidly on a chainomatic analytical balance. The fungus used, *Rhizopus suinus* Niels., was isolated by CHRISTIANSEN (3) and described by NIELSEN (9).

## Experimentation

Table 1 gives the data for the first series of cultures with the extracted dextrose. It may be seen that extraction of dextrose with alcohol and ether results in the removal from the sugar of a substance or substances which are in some manner growth inhibiting. Further, a preliminary extraction with acetone removes an

additional amount of the substance or substances, or some other substance. These results were confirmed in a second series of cultures, this time involving only two different concentrations of dextrose. Each figure for the average mat weight in table 2 is the average obtained from twenty-four cultures.

The data in table 3 and figure 1 show that extraction of the galactose and dextrose causes considerable increase in the weight of the mycelium of *R. suinus*

TABLE 1

EFFECT OF ALCOHOL-ETHER AND ACETONE-ALCOHOL-ETHER EXTRACTIONS OF DEXTROSE ON GROWTH OF RHIZOPUS SUINUS

| Concentration of dextrose | Average mat weight (mg.) | | | Percentage of control | |
|---|---|---|---|---|---|
| | Unextracted | Alcohol-ether extracted | +Acetone treatment | Alcohol-ether treated | Acetone-alcohol-ether treated |
| 0.005M..... | 18 | 19 | 18 | 106 | 100 |
| 0.01M..... | 25 | 26 | 28 | 104 | 112 |
| 0.02M..... | 39 | 44 | 42 | 113 | 108 |
| 0.03M..... | 49 | 52 | 58 | 106 | 118 |
| 0.05M..... | 56 | 63 | 73 | 113 | 130 |
| 0.10M..... | 61 | 68 | 106 | 112 | 174 |
| 0.20M..... | 79 | 98 | 138 | 124 | 174 |

TABLE 2

EFFECT OF EXTRACTION OF DEXTROSE ON GROWTH OF RHIZOPUS SUINUS

| Concentration of dextrose | Average mat weight (mg.) | | | Percentage of control | |
|---|---|---|---|---|---|
| | Untreated | Alcohol-ether | Acetone-alcohol-ether | Alcohol-ether | Acetone-alcohol-ether |
| 0.02M..... | 50 | 55 | 56 | 110 | 112 |
| 0.20M..... | 89 | 124 | 138 | 140 | 156 |

which develops on the culture medium containing these sugars. In the extracted as well as in the unextracted state, galactose appears to be definitely superior to dextrose as a source of carbon for this fungus. HORR (7), EDGECOMBE (4), and STEINBERG (12), however, state that galactose is a poor source of carbon for fungi.

Table 4 combines the results of several culture series. Only three concentrations of each sugar were used in these experiments. Enough of each sugar was extracted to provide sufficient material for the experiments cited in the table. Each figure for the mat weight is the average for twelve individual cultures.

TABLE 3

EFFECT OF EXTRACTION OF DEXTROSE AND GALACTOSE ON RELATIVE GROWTH OF RHIZOPUS SUINUS ON THE TWO SUGARS

| Concentration of sugar | Average mat weight (mg.) | | | | Treated dextrose as percentage of untreated | Treated galactose as percentage of untreated |
|---|---|---|---|---|---|---|
| | Untreated dextrose | Treated dextrose | Untreated galactose | Treated galactose | | |
| 0.02M..... | 43 | 43 | 43 | 60 | 100 | 140 |
| 0.03M..... | 59 | 61 | 60 | 75 | 103 | 125 |
| 0.04M..... | 64 | 72 | 72 | 96 | 113 | 133 |
| 0.05M..... | 67 | 82 | 88 | 119 | 122 | 135 |
| 0.10M..... | 77 | 104 | 99 | 166 | 135 | 136 |
| 0.15M..... | 82 | 124 | 112 | 182 | 151 | 162 |
| 0.20M..... | 85 | 135 | 126 | 197 | 159 | 156 |

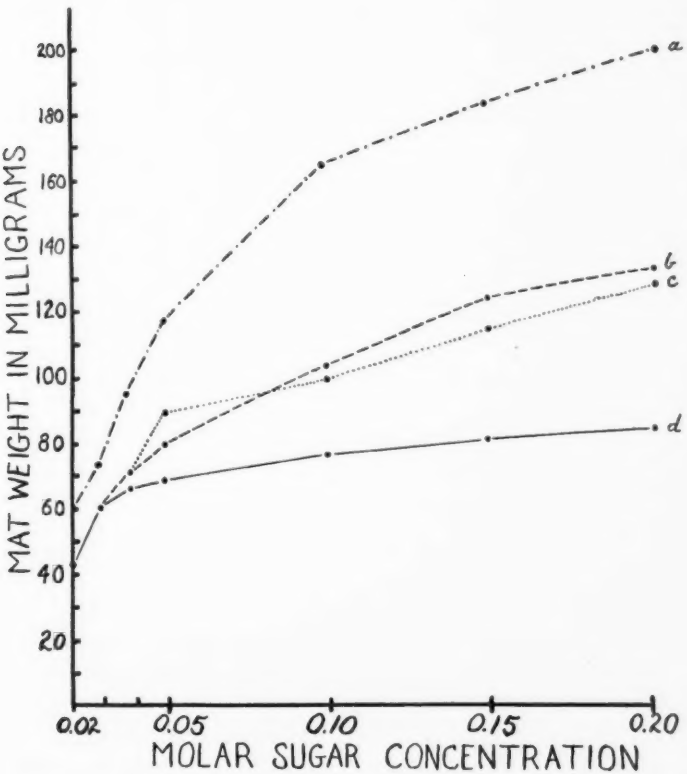


FIG. 1.—Comparison of growth of *Rhizopus suinus* on treated and untreated dextrose and galactose: *a*, treated galactose; *b*, treated dextrose; *c*, untreated galactose; *d*, untreated dextrose.

In all the cases studied, extraction of the sugars resulted in increased growth of *R. suinus* on the extracted sugar. The greatest increase was with levulose, where

TABLE 4

EFFECT OF EXTRACTION OF DEXTROSE, SUCROSE, AND LEVULOSE ON GROWTH OF RHIZOPUS SUINUS ON THESE SUGARS

| SUGAR | NO. OF SERIES* | CONCENTRATION | AVERAGE MAT WEIGHT (MG.) | | PERCENTAGE UNTREATED (CONTROL) |
|---|---|---|---|---|---|
| | | | UNTREATED | TREATED | |
| Dextrose | 1 | 0.05M | 124 | 141 | 114 |
| | 2 | 0.05 | 96 | 107 | 113 |
| | 3† | 0.05 | 78 | 90 | 116 |
| | 4 | 0.05 | 72 | 82 | 114 |
| | 5 | 0.05 | 84 | 94 | 112 |
| | 1 | 0.10 | 156 | 173 | 111 |
| | 2 | 0.10 | 113 | 132 | 117 |
| | 3† | 0.10 | 103 | 118 | 115 |
| | 4 | 0.10 | 98 | 116 | 118 |
| | 5 | 0.10 | 118 | 139 | 118 |
| | 1 | 0.20 | 172 | 192 | 112 |
| | 2 | 0.20 | 131 | 148 | 112 |
| | 3† | 0.20 | 135 | 148 | 110 |
| | 4 | 0.20 | 121 | 135 | 111 |
| | 5 | 0.20 | 140 | 160 | 119 |
| Sucrose | 1 | 0.05 | 15 | 17 | 114 |
| | 2 | 0.05 | 12 | 13 | 111 |
| | 1 | 0.10 | 40 | 47 | 118 |
| | 2 | 0.10 | 34 | 41 | 120 |
| | 1 | 0.20 | 80 | 92 | 115 |
| | 2 | 0.20 | 87 | 96 | 111 |
| Levulose | 1 | 0.05 | 73 | 103 | 142 |
| | 2 | 0.05 | 67 | 78 | 115 |
| | 1 | 0.10 | 99 | 127 | 128 |
| | 2 | 0.10 | 99 | 115 | 116 |
| | 1 | 0.20 | 149 | 188 | 126 |
| | 2 | 0.20M | 131 | 161 | 123 |

* Individual series with corresponding numbers made at same time and inoculated from same spore suspension.

† Recrystallized from 80% alcohol, rather than extracted.

the average increase over the control was 22–28.5 per cent. Judging from its appearance, levulose was the most impure of the sugars.

The sugar used in series 3 was purified differently from that in the other four series. The dextrose was dissolved in as small a volume of hot water as possible,

and enough 95 per cent alcohol was added to make the final concentration 80 per cent. The solution was then stored in a cold place for 24 hours, during which time crystallization of the sugar took place. The supernatant alcohol was discarded, and the dextrose was dried. The results were not appreciably different from those obtained by extraction with the various solvents.

Since extraction or recrystallization of dextrose and other sugars resulted in an increased growth of *R. suinus* in the culture series in which the treated sugar served as the carbon source, it is evident that in the extract there must be a substance or complex of substances that retard assimilation of the sugar. Several attempts were made to determine the nature of this material. The first attempt was to obtain the extracted substance in a concentrated form. The extracts from 100 gm. of dextrose (1000 cc. acetone, 1000 cc. alcohol, and 500 cc. ether) were

TABLE 5

EFFECT OF ADDITION OF VARIED AMOUNTS OF DEXTROSE EXTRACT TO CULTURE MEDIUM UPON GROWTH OF RHIZOPUS SUINUS

| AMOUNT OF EXTRACT (CC.) | AVERAGE MAT WEIGHT (MG.) | PERCENTAGE OF CONTROL | AMOUNT OF SPORE FORMATION |
|---|---|---|---|
| 0 | 74.6 | 100 | Slight |
| 1 | 105.9 | 142 | Slight |
| 3 | 139.8 | 187 | Moderate |
| 5 | 153.8 | 206 | Heavy |

evaporated to a small volume (100 cc. each) over a steambath and stored in a cold place until a large part of the dissolved sugar had crystallized. The three supernatant liquids were then combined and evaporated to dryness over the steambath. The dry residue was made up to 100 cc. in distilled water, so that each cubic centimeter of the extract contained the substances derived from 1 gm. of the sugar. A culture series containing 0.05*M* extracted dextrose, with from none to 5 cc. of the extract added, was inoculated with a spore suspension of *R. suinus*. Ten replicates of each concentration of the extract were used. The results are shown in table 5.

From the behavior of the fungus with respect to spore formation when different amounts of the extract were added to the culture medium, it was thought possible that at least one of the substances present in the extract was vitamin $B_1$ (thiamin). This supposition was based upon the behavior of agar plate cultures of *R. suinus* when synthetic vitamin $B_1$ was added to the medium. This work is to be discussed in detail in a forthcoming paper.

BARGER, BERGEL, and TODD (**2**) and JANSEN (**8**), among others, have shown that cautious oxidation of vitamin $B_1$ yields a yellow pigment, thiochrome, which shows

a blue fluorescence under ultraviolet light. A qualitative method for the determination of the presence of vitamin $B_1$ was used as follows, based on the method of BARGER *et al.*, for the formation of thiochrome.

To a solution of vitamin $B_1$ hydrochloride in 1–2 cc. of alcohol is added 2 cc. of 15 per cent potassium hydroxide in methyl alcohol and 1 cc. of an aqueous potassium ferricyanide solution. To this mixture is added 10 cc. of butyl alcohol, and the whole is shaken for about 2 minutes. The yellow pigment is separated in the butyl-alcohol layer and may then be tested for ultraviolet fluorescence.

Using synthetic vitamin $B_1$ in ethyl-alcohol solution, a fairly strong fluorescence was observed when 1 cc. of a 0.01 per cent solution of thiamin (0.1 mg.) was used. The oxidation was tried on the water solution of the dextrose extract, but the results were doubtful, since any fluorescence present was weak, and at the time of this particular experiment, suitable filters were not available.

The original methods of BARGER *et al.* and of JANSEN call for the use of a methyl-alcohol solution of the vitamin or of the material suspected to contain the vitamin. To obtain a critical test for the presence of vitamin $B_1$ in the sugar, a 100-gm. sample of dextrose was treated for several hours in a Soxhlet extractor with absolute methyl alcohol. The extract was then evaporated over a steambath to about 25 cc. and allowed to cool. A large part of the sugar dissolved by the extraction process crystallized out. The supernatant liquid was further concentrated to 10 cc.

Oxidation of the extract with alkaline potassium ferricyanide was carried out as already described. The resulting butyl-alcohol layer showed a yellow coloration, so that it was necessary to confirm the results by demonstration of ultraviolet fluorescence. The source of the ultraviolet was an 85-watt quartz mercury vapor lamp. In the opening in the lamp housing was placed a Corning 597 filter for transmitting the ultraviolet. When a Corning Noviol-A filter was superimposed upon the 597 filter, practically no light was transmitted. When the two filters were separated and the tube containing the yellow-colored butyl-alcohol layer was placed between them, a distinct blue color was observed through the Noviol filter, demonstrating the presence of thiochrome. Vitamin $B_1$ is therefore present in the reagent grade dextrose.

ROBBINS and KAVANAGH (10) showed that *Rhizopus nigricans*, both the plus and minus strains, grew satisfactorily in a liquid medium of mineral salts, asparagine, and dextrose, but its growth was materially reduced by the addition of 1 p.p.m. of vitamin $B_1$. SCHOPFER (11) had previously shown that various *Rhizopus* species, including *R. suinus*, showed reduction of growth when small amounts of vitamin $B_1$ were added to the culture medium. This line of evidence is consistent with the present finding that extraction of the sugars in the manner described (with consequent removal of traces of thiamin) results in increased growth of the fungus on the extracted sugar. In table 5, however, it may be seen that growth of

the fungus is roughly proportional to the amount of the concentrated extract added to the culture medium. A further extensive culture series was set up in order to confirm these rather striking results. A second dextrose extract was prepared and evaporated to dryness. To be sure that the increased vegetative growth upon addition of the extract was not a result of the dextrose contained in the extract, two precautions were taken. The sugar concentration of the medium was increased to 0.10*M*, and sets of cultures were used—each having an amount of 1.25 per cent dextrose solution added equal to the amounts of the dextrose extract used. The weight of the evaporated dextrose extract was 1.25 gm., and this was made up to 100 cc. with distilled water. Culture flasks were also prepared in which the culture

TABLE 6

EFFECT OF DEXTROSE EXTRACT AND OF VITAMIN $B_1$ ON GROWTH OF RHIZOPUS SUINUS ON DEXTROSE

| TREATMENT | AVERAGE MAT WEIGHT (MG.) | PERCENTAGE OF CONTROL | PERCENTAGE OF CULTURES WITH 1.25% DEXTROSE SOLUTION ADDED | SPORE FORMATION |
|---|---|---|---|---|
| Extracted dextrose | 77.5 | 100 | ..... | Weak |
| +1 cc. extract | 123.0 | 160 | 153 | Weak |
| +3 cc. extract | 195.4 | 252 | 202 | Moderate |
| +5 cc. extract | 219.9 | 284 | 227 | Heavy |
| +1 p.p.b. thiamin | 69.5 | 90 | ..... | Moderate |
| +1 p.p.m. thiamin | 56.9 | 73 | ..... | Moderate |
| +1 cc. 1.25% dextrose | 80.6 | 104 | ..... | Weak |
| +3 cc. 1.25% dextrose | 96.5 | 125 | ..... | Weak |
| +5 cc. 1.25% dextrose | 96.7 | 125 | ..... | Weak |

medium contained 1 p.p.m. and 1 p.p.b. of vitamin $B_1$. The results of this experiment are shown in table 6. The mat weight figures are the averages of ten cultures.

The concentrated extract of dextrose is shown to have great influence on vegetative growth and spore formation. Vitamin $B_1$ has a mild growth-retarding effect and a strong favorable influence on the formation of spores. Finally, in the cultures to which the 1.25 per cent sugar solutions were added, only a small part of the growth increase caused by the dextrose extract could be a result of the actual dextrose present in the extract. In an attempt to learn something of the nature of the growth-promoting material in the dextrose extract, the following procedure was adopted:

One hundred gm. of dextrose was ashed. The small amount of ash was dissolved in a small amount of hydrochloric acid, neutralized with sodium hydroxide with phenolphthalein as the indicator, and made up to 100 cc. with distilled water.

A culture series was set up, with 0.10*M* dextrose as the carbon source, and different amounts of the solution of ash elements were added to the medium. Ten replicates of each concentration were used.

Table 7 indicates that there are present in reagent-grade dextrose nonvolatile constituents (heavy metals) whose presence results in increased dry weight production by *R. suinus*. Possibly the growth relations of this fungus upon dextrose may be summarized in the following manner:

Reagent-grade dextrose of the brand used in these experiments contains traces of vitamin $B_1$ and small amounts of ash elements. The demonstration of other possible organic impurities is a matter for future consideration. The mineral elements cause increased growth of *R. suinus*, while vitamin $B_1$ causes decreased growth and

TABLE 7

EFFECT OF ADDITION OF DEXTROSE TO CULTURE MEDIUM UPON GROWTH OF RHIZOPUS SUINUS ON DEXTROSE

| AMOUNT OF ASH SOLUTION ADDED | AVERAGE MAT WEIGHT (MG.) | PERCENTAGE OF UNTREATED CONTROL | SPORE FORMATION |
|---|---|---|---|
| 0 | 100 | 100 | Slight |
| 1 | 144 | 144 | Slight |
| 3 | 169 | 169 | Slight |
| 5 | 198 | 198 | Slight |

increased spore formation. It is suggested that the increase in growth on the dextrose which has been extracted with acetone, alcohol, and ether may be explained on the basis of the following facts. Removal of the vitamin $B_1$ eliminates the growth-retarding effect which has been demonstrated by ROBBINS and KAVANAGH, SCHOPFER, and the writer. This removal tends to cause increase in growth. The absence of spore formation would mean that a certain amount of energy and material, normally utilized in the production of spores, could be utilized in vegetative growth. This would counteract the unfavorable effect of the extraction process on growth of the fungus resulting from removal of heavy metals from the sugar. Concentration of the active agents which follows with concentration of the dextrose extract probably influences the effect of the heavy metals more than the effect of the vitamin $B_1$. Thus, when the concentrated extract is added to a culture, the effect of the heavy metals present more than balances the effect of the thiamin, and the result is augmentation of growth. Possibly the growth-retarding effect of vitamin $B_1$ is a corollary of the tendency of the vitamin to predispose the fungus to spore formation.

**Summary**

1. Extraction of dextrose, levulose, galactose, and sucrose with acetone, alcohol, and ether resulted in increased mycelial production by *Rhizopus suinus*.

2. Extraction of dextrose with alcohol and ether resulted in increased mycelial production by this fungus. The increase was not so great as with the acetone-alcohol-ether extraction.

3. Recrystallization of dextrose from 80 per cent alcohol resulted in growth augmentation of about the same magnitude as that which resulted from the acetone-alcohol-ether treatment.

4. The presence of vitamin $B_1$ (thiamin, aneurin) in reagent-grade dextrose was shown by the thiochrome oxidation method. Removal of traces of vitamin $B_1$ from the sugars during the extraction process is possibly the cause of the growth phenomena.

5. The extract of dextrose contains a substance which, when concentrated by evaporation, promotes the vegetative growth of *R. suinus* if it is added to the culture medium. The substance was demonstrated, by growth experiments in which the nutrient solution was supplemented with the ash obtained from the unpurified dextrose, to be an ash constituent.

6. Under the conditions of the extraction, the removal of vitamin $B_1$ would be more extensive than the removal of the ash constituents, the influence of the removal of the thiamin consequently being more effective in increasing the growth of *R. suinus* than the removal of the small amount of ash constituents in decreasing the growth of the organism. When the extract is concentrated, however, the influence of the ash element(s) on mycelial production is much greater than the influence of the vitamin $B_1$, and the result is growth augmentation.

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LITERATURE CITED

1. ALLISON, F. E., and HOOVER, S. R., An accessory factor for legume nodule bacteria. Sources and activity. Jour. Bact. **27**:561–581. 1934.
2. BARGER, G., BERGEL, F., and TODD, A. R., Ueber das Thiochrom aus Vitamin $B_1$ (Antineurin). Ber. Deutsch. Chem. Ges. **68**:2257–2262. 1935.
3. CHRISTIANSEN, M., Mucormykose beim Schwein. I. Mitt. Virchow's Arch. Path. Anat. Phys. **273**:829–858. 1929.
4. EDGECOMBE, A. E., The effect of galactose on the growth of certain fungi. Mycologia **30**: 601–624. 1938.

5. FUNK, C., and FREEDMAN, L., The presence of a yeast growth promoting vitamine in cane sugar. Jour. Biol. Chem. **56**:851–860. 1923.
6. HALL, H. H., JAMES, L. H., and STEWART, L. S., Yeast growth stimulants in white sugars. Indust. Eng. Chem. **25**:1052–1054. 1933.
7. HORR, W. H., Utilization of galactose by *Aspergillus niger* and *Penicillium glaucum*. Plant Physiol. **11**:81–99. 1936.
8. JANSEN, B. C. P., A chemical determination of aneurin (vitamin $B_1$) by the thiochrome reaction. Rec. Trav. Chim. Pays-bas. **55**:1046–1052. 1936.
9. NIELSEN, N., Mucormykose beim Schwein. II. Mitt. Beschreibung der isolierten Pilze. Virchow's Arch. Path. Anat. Phys. **273**:859–863. 1929.
10. ROBBINS, W. J., and KAVANAGH, F., Vitamin $B_1$ or its intermediates and the growth of certain fungi. Amer. Jour. Bot. **25**:229–236. 1938.
11. SCHOPFER, W. H., Etude de l'action du saccharose à divers états de pureté. Ann. Mycol. **33**:28–32. 1935.
12. STEINBERG, R. A., Growth of fungi in synthetic nutrient solutions. Bot. Rev. **5**:1–44. 1939.
13. ———, Relation of accessory growth substances to heavy metals, including molybdenum, in the nutrition of *Aspergillus niger*. Jour. Agr. Res. **52**:439–448. 1936.
14. WILDIERS, E., Nouvelle substance indispensable au développement de la levure. La Cellule **18**:313–336. 1901.

# FOREST SEQUENCES IN THE NORTH CENTRAL STATES

PAUL B. SEARS

(WITH SIX FIGURES)

## Introduction

This paper presents fifteen pollen profiles from as many peat deposits in Illinois, Indiana, Michigan, and Ohio, only two of which—Bucyrus and Mud Lake (Ohio)—have been previously published. These profiles have been prepared to show only the relative fluctuations of eight forest genera and one family, Betulaceae.

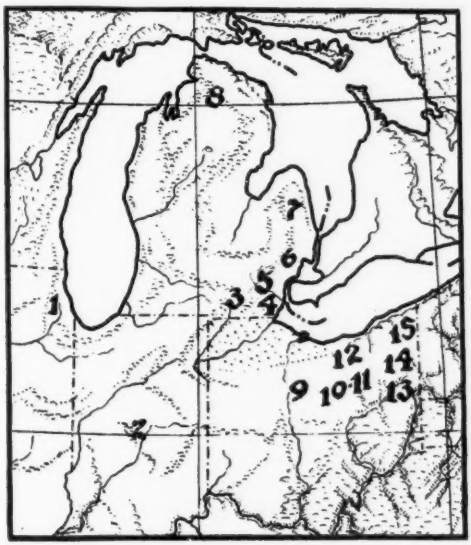


FIG. 1.—Location of bogs studied

The location of these deposits is indicated on the map (fig. 1) and again on each legend (figs. 2–6). Depth of samples analyzed is indicated by solid bars in each column, and by depth scale at the left. Length of bars represents relative percentages; but for more graphic comparison, bars are centered on scale instead of starting from zero point. In each graph, Roman numerals indicate corresponding stages.

A number of the profiles have been truncated by fire and cultivation. Others have been condensed because of drainage. Sedimentation began much earlier in some than in others, as is evident by noting positions relative to glacial retreat.

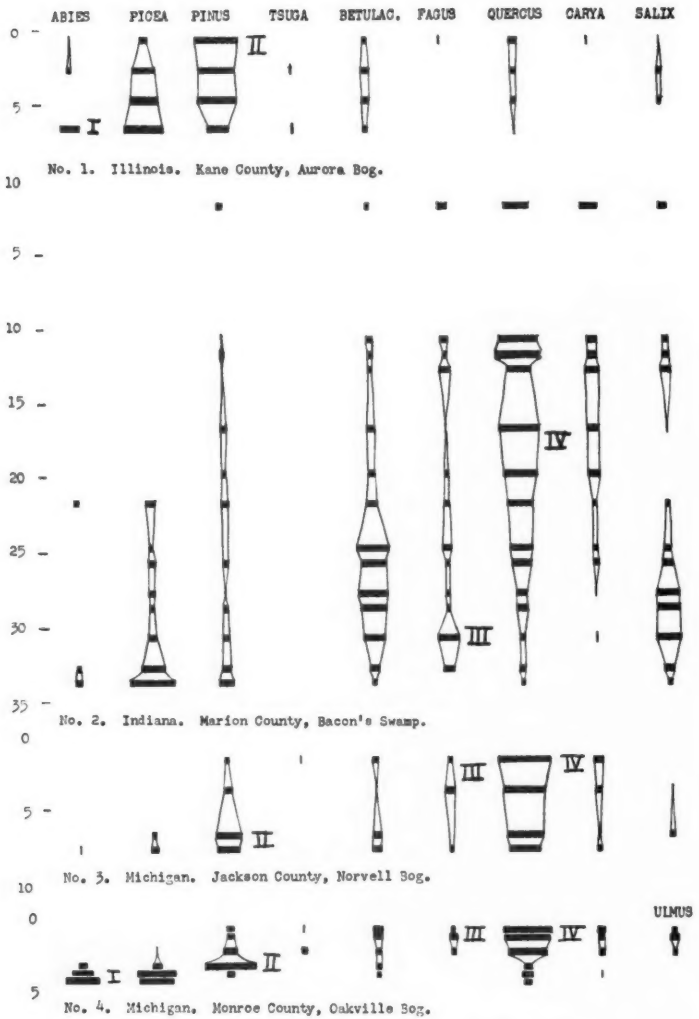


FIG. 2.—Pollen profiles of important forest components, bogs 1–4

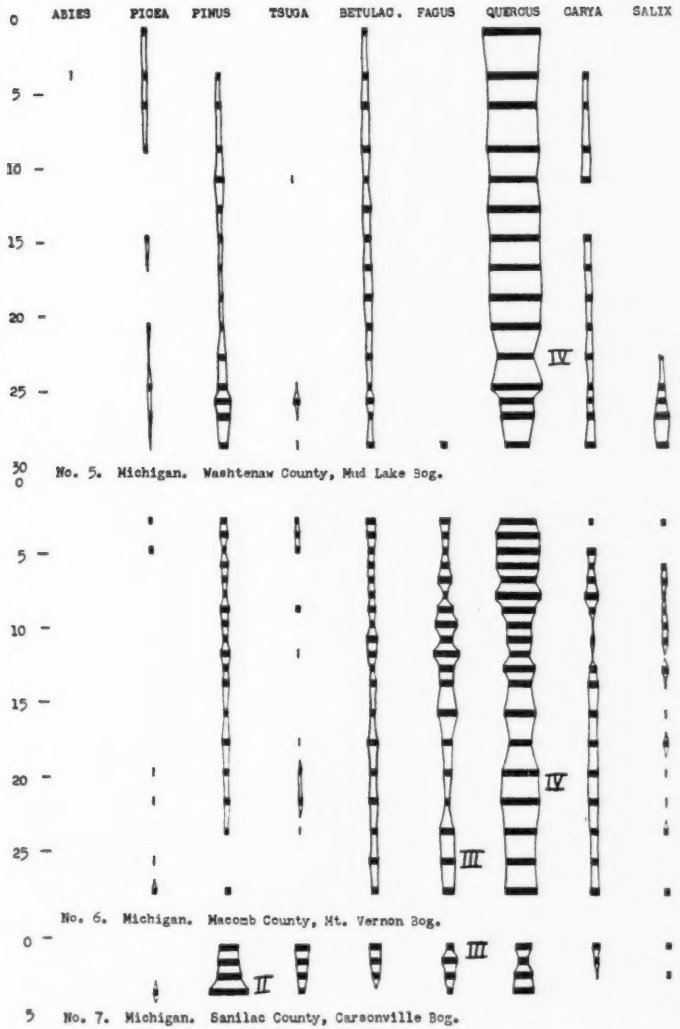


FIG. 3.—Pollen profiles of important forest components, bogs 5–7

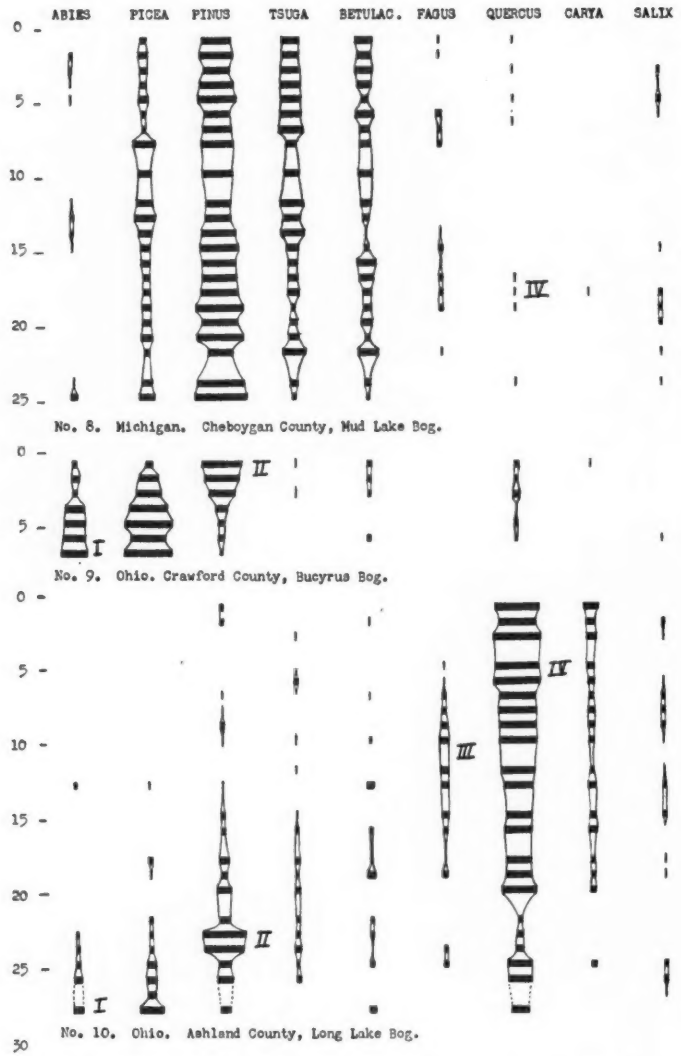


FIG. 4.—Pollen profiles of important forest components, bogs 8–10

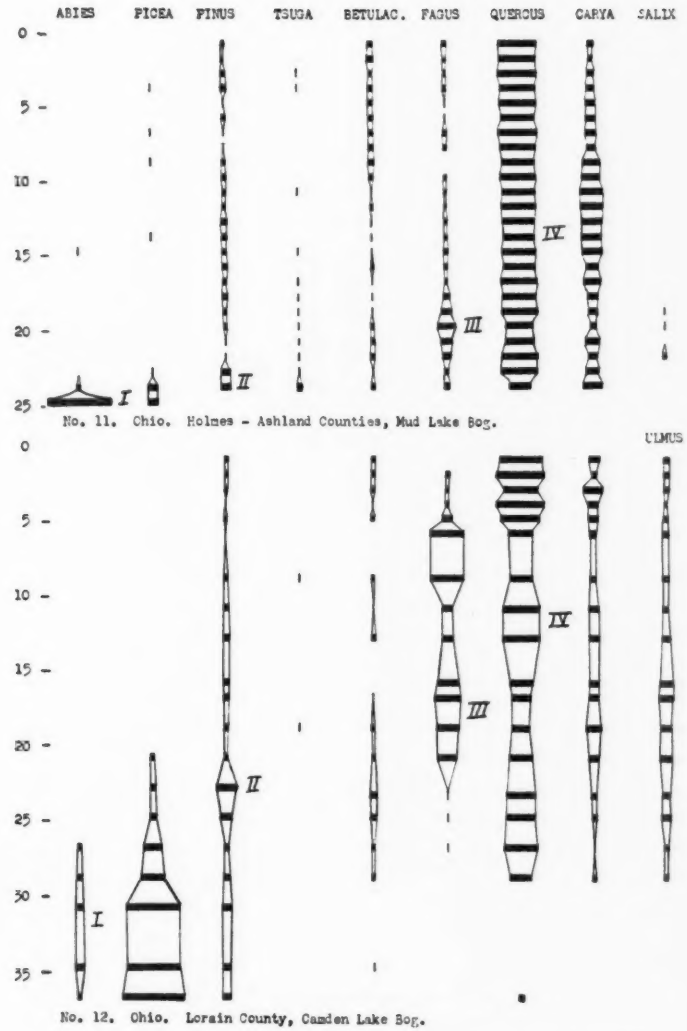


FIG. 5.—Pollen profiles of important forest components, bogs 11 and 12

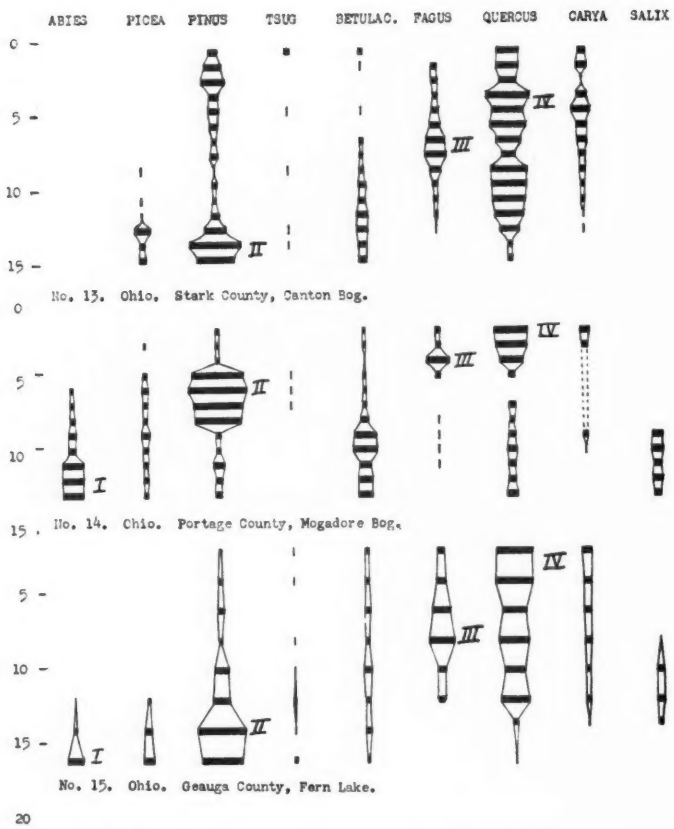


FIG. 6.—Pollen profiles of important forest components, bogs 13–15

### Observations

1. Aurora Bog.—This profile was secured from material in which mastodon remains were found. It represents an early segment of postglacial time and shows a striking increase of pine at the expense of fir and spruce.

2. Bacon's Swamp.—This profile shows a brief maximum of beech following spruce and pine. Beech gives way to birch-oak, followed by oak-hickory.

3. Norvell Bog.—This profile shows pine reaching a maximum, then beech, followed by oak-hickory. The top of this deposit has been destroyed.

4. Oakville Bog.—This is an old deposit, truncated and condensed. The shift from fir and spruce to a sharp pine maximum is evident. The next shift, from pine to oak, is marked by a faint interval during which beech is at a maximum, although never abundant. Elm behaves like beech.

5. Mud Lake (Washtenaw) Bog.—This is essentially post-coniferous. The presence of beech and hemlock at the bottom, with oak minimum, probably corresponds to the end of early beech maximum noted elsewhere.

6. Mt. Vernon Bog.—This is likewise post-coniferous. Early and later beech shows maxima, inversely correlated with oak and hickory.

7. Carsonville Bog.—This condensed deposit has been severely burned and drained. It begins with pine and oak maxima, followed by a beech maximum which is temporary. Beech yields to oak and hickory; but pine, birch, and hemlock remain as important constituents.

8. Mud Lake (Cheboygan) Bog.—This corresponds to a deciduous period farther south. Spruce, pine, hemlock, and birch are found throughout. Temporary displacement of hickory, oak, and beech by spruce and hemlock is notable, near the middle of the profile. In position this corresponds to the second (upper) beech maximum shown in certain other profiles. It suggests cooling.

9. Bucyrus Bog.—This has been previously published (**5**). It shows in striking fashion the shift from fir and spruce to pine evident in the other early profiles of this report.

10. Long Lake Bog.—Here is a sharply marked pine maximum, following the waning of fir and spruce, and some distance above it a definite maximum of beech, replaced by oak and hickory toward the top.

11. Mud Lake (Ohio) Bog.—This has been previously published (**6**). At the base is a telescoped record of shift from fir and spruce to pine, oak, and hickory. Above this is a brief but striking maximum of beech, giving way to an equally striking but more prolonged dominance of oak and hickory. In the original report the waning of hickory near the top and the increase of other species, not here included, was interpreted as an increase of mesophytism in recent times. In view of

the other profiles here presented, however, it may be questioned whether this was justified.

12. CAMDEN LAKE BOG.—Here the pine maximum, marked by disappearance of fir and waning of spruce, is definite. Above it is the beech maximum, characteristic elsewhere and followed by a maximum of oak and hickory. Above this the cycle is again repeated, with a second beech maximum, followed once more by oak and hickory.

13. CANTON BOG.—This is very near the glacial limit. No fir is showing, but pine-oak maximum, followed in order by beech, oak, and hickory. The top of this deposit has been cultivated and doubtless also burned.

14. MOGADORE BOG.—The top is likewise destroyed, but the shift from fir to pine maximum is definite; likewise subsequent beech maximum, followed by oak and hickory.

15. FERN LAKE BOG.—Pine-oak maximum is very definite, with subsequent beech maximum, giving way to oak and hickory.

## Discussion

Bog 8 lies in a region of coniferous forest. The remainder are in a region now characterized by deciduous forest—save no. 7, which is intermediate in position. Records of a formerly coniferous forest are present in all but nos. 5 and 6. In every instance this coniferous period was terminated by a strongly marked pine maximum. This is least marked in nos. 2 and 11. There is a concurrence of oak (often with hickory) and pine in nos. 3, 4, 7, 11, 12, 13, 15, and it is suggested in other profiles.

The pine maximum develops at the expense of fir in nos. 1, 4, 9, 10, 11, 12, 14, and 15; at the expense of spruce in nos. 1, 3, 4, 9, 10, 11, 12, and 15. The conclusion seems warranted that fir and spruce forests were replaced by pine. Spruce appears to bridge the transition from fir to pine. Generally speaking, this suggests a lowering of the degree of mesophytism. The usual order of succession is from pine to fir and spruce. This interpretation is strengthened by two circumstances: (*a*) the concurrence of pine and oak noted in the preceding paragraph and (*b*) the fact that the first pines to be represented are *Pinus banksiana* and *P. resinosa*. *P. strobus*, the most mesophytic, is most abundant toward the end of the pine maximum in the profiles studied. This is not shown in the diagrams.

Following the pine there is an increase of beech in nos. 2, 3, 4, 6, 7, 10, 11, 12, 13, 14, and 15. No. 1 shows the first appearance of beech at the pine maximum, but the record is truncated above that level. No. 5 begins above the pine maximum, and the only beech in the profile is at the base. No. 8 is not comparable, being in the coniferous region, while no. 9 has no beech and is truncated below the level at which beech probably first appeared. In every instance except these four,

the beech increases to a maximum, then decreases in favor of oak and hickory. The behavior of beech suggests an increase of mesophytism, followed by a lessening similar to that involved in the shift from fir and spruce to pine.

Profiles 2, 6, and 12 show a second maximum of beech above the first. But at the top, all profiles extending through deciduous time (nos. 2, 5, 6, 10, 11, 12, and 13) agree in showing a dominance of oak and hickory over beech. So far as beech indicates mesophytism, the present or recent past is a time of reduced mesophytism. Here again no. 8 is regarded as anomalous, being in a different environment.

COWLES (3) employed the term retrogression to designate a lowering of the level of mesophytism in a community. While chiefly concerned with this phenomenon in relation to physiographic change, he pointed out that it may be due to other factors and specifically mentions climatic change as one. The normal course of succession he recognized as being toward the greatest degree of mesophytism possible under existing conditions. Other terms applied to this phenomenon are regression, rejuvenation, and degeneration. The term employed by COWLES has the advantage of priority and clarity of definition. For this reason, although the writer is familiar with criticisms of the concept (2), he agrees with CAIN (1) in accepting the term.

The preceding discussion may therefore be summarized in the statement that twice during postglacial times there has occurred a retrogression in forest composition in the north central states. The first was a shift from fir through spruce to pine; the second a shift from beech to oak and hickory. Three of the seven profiles which extend to the present show a second appearance of beech followed by a third retrogression. Whether this third retrogression was due to local causes, or to general causes whose operation was buffered in four of the seven profiles which extend through deciduous time, is not clear.

In the observed course of plant succession, the movement is from less to more mesophytism. Relative stability is reached when the mesophytes present are able to reproduce themselves under conditions of their own domination. It follows that, if vegetation is replaced by that which is less mesophytic, a disturbance of the normal course of succession has taken place. Such a disturbance is implied in the two rather general instances of retrogression just described. Three obvious factors are involved in such a change. These are space, light, and moisture balance. Unless new areas are being opened up to vegetation in the vicinity, a pollen profile that shows a shifting vegetation must indicate that the new kind or kinds of vegetation are using the physical space occupied by the old.

In general, the scale of increasing mesophytism is paralleled by an increasing shade tolerance, or a decreasing light requirement. So far as this is true, the possi-

bility of retrogression would appear to be limited so long as the mature dominant mesophytes remain alive and in place, whether they are reproducing or not.

Both of these circumstances suggest strongly that retrogression from spruce and fir to pine, and later from beech to oak and hickory, must have been subsequent to the dying out, or killing out, of the more mesophytic forms.

It is not easy to postulate any biotic process or physiographic change so general in character as to account for such destruction and subsequent retrogression in the numerous localities involved. On the other hand, a less favorable water balance might produce just this result. Beech is notably sensitive to drought. Apparently the most reasonable explanation of the observed retrogression lies along this line.

The postglacial history of the Great Lakes is well known and has been marked by lowering of the drainage level. Conceivably this might have resulted in periods of rather general lowering of the water table. In view, however, of the variety of secondary drainage basins involved, the general low relief of the region, its generally adequate soil moisture before settlement, and particularly the continuous sedimentation within the basins studied, it may be doubted whether such recession of lake level would explain the retrogressions. On the other hand, these would be satisfactorily explained by periods of reduced humidity. This explanation is consistent with the presence of relict communities indicating one or more periods of relative dryness in postglacial times (**4**). And it agrees with the interpretation of Mud Lake (Ohio) profile (**6**). In this paper the writer classified the plants whose pollen was found on the basis of their supposed climatic significance. The conclusion was reached that there had been two periods of relative dryness, corresponding to those inferred from the present account of retrogressions.

In another paper (**7**) the writer compared the types of pollen profiles from various parts of eastern North America. With the exception of no. 8, those discussed in the present paper represent the "deciduous forest, central type" of the earlier report.

On the whole, the bogs of this region show little evidence of recent "climatic deterioration" in the sense of cooling and increased humidity following a postglacial warm, dry, or xerothermic period. The evidence of this is much clearer in the coniferous region bogs extending from Minnesota toward the Atlantic, where a maximum of oak is evident about mid-profile. This xerothermic oak maximum may correspond to the 18-foot level of our present no. 8 in northern Michigan.

The presence of "relict" species and communities, notably prairie, in the central deciduous area was responsible for the original hypothesis of a xerothermic period in North America. There has been no reason to assume that these western relicts were expanding aggressively at the time of European settlement. There is evidence to the contrary (**4**). So far as it has been studied, the general trend of plant succession in this region is normal—toward increased mesophytism. This

may have been accelerated by the cooling and increasing humidity evident farther north. Or it may merely represent ecological recovery following the shock of the second retrogression evident in the present data. In neither case does it appear to have produced any striking effect in the profiles here considered. More detailed study of profiles which are intact down to the present may shed light on this problem. Meanwhile, the two periods of forest retrogression described appear adequate to explain the presence in the central deciduous region of species and communities of more continental type than is now prevalent there.

## Summary

1. Fifteen pollen profiles from bogs in the states of Illinois, Indiana, Michigan, and Ohio are figured, thirteen of which have not before been published. All but one are in the central deciduous region.

2. The forest sequence in these profiles shows two periods of retrogression. The earlier was a retrogression from fir and spruce to pine. This was followed by a relative increase of beech, ending in a second retrogression, when oak and hickory increased at the expense of beech. Three of the profiles show subsequent increase and retrogression of beech, but this does not appear to have been general.

3. The two general periods of retrogression are assumed to have been due to climatic causes, producing a less favorable water balance. This is considered adequate to explain the presence of xerothermic relicts in the central deciduous region.

The analysis of Fern Lake Bog by EVA GERSBACHER and of Camden Lake Bog by PRESTON SMITH were thesis problems. Much of the field and laboratory work was made possible by grants-in-aid from the Carnegie Institution of Washington.

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## LITERATURE CITED

1. CAIN, S. A., The climax and its complexities. Amer. Mid. Nat. **21**:146–181. 1939.
2. CLEMENTS, F. E., Plant succession. Carnegie Inst. Washington. 1916.
3. COWLES, H. C., The physiographic ecology of Chicago and vicinity: A study of the origin, development and classification of plant societies. BOT. GAZ. **31**:73–108; 145–182. 1901.
4. GLEASON, H. A., The vegetational history of the middle west. Ann. Assoc. Amer. Geog. **12**:39–85. 1923.
5. SEARS, P. B., A record of post-glacial climate in northern Ohio. Ohio Jour. Sci. **30**:205–217. 1930.
6. ———, Pollen analysis of Mud Lake Bog in Ohio. Ecology **12**:650–655. 1931.
7. ———, Types of North American pollen profiles. Ecology **16**:488–499. 1935.

# BEHAVIOR OF WOODY DESERT LEGUMES AT THE WILTING PERCENTAGE OF THE SOIL

R. F. DAUBENMIRE AND H. E. CHARTER

(WITH THREE FIGURES)

## Introduction

According to many students of soil-plant relationships at the wilting percentage of the soil, the most drought-resistant of desert plants as well as the most drought-sensitive mesophytes should face a physiological crisis in their water economy at this point. In most mesophytes, such a crisis is easily recognized by a wilting of the leaf blades; but in many xerophytes, especially in sclerophyllous and succulent species, this usual manifestation of loss of turgor is lacking and others must be sought. Certain—if not all—of the woody desert legumes of the North American deserts are among this group of plants not exhibiting the usual symptoms of wilting.

ALWAY (1) planted woody desert legumes in metal containers, and after the plants were well established irrigation was withheld for a long period. He observed that *Prosopis velutina*, *Acacia greggii*, and *A. constricta* remain alive long after the soil moisture drops below the wilting percentage, and that during such protracted drought the tips of the shoots died, the lower leaves abscissed, growth ceased, and the transpiration rate declined. In none of his experiments, however, did he attempt to correlate the relationship of these responses with the attainment of the wilting percentage of the soil and thus determine whether this point is of any more significance to these plants than are lower moisture percentages. It was with this question in mind that the experiments reported here were undertaken.

## Material and methods

Palouse silt loam, into which was thoroughly mixed a small proportion of sand, was used throughout the experiments, and quart-capacity motor-oil tins were used for soil containers ("pots"). Before planting, the leguminous seeds were either notched with a file or treated with sulphuric acid, and with such treatments they germinated promptly. Three species of legumes were used: *Prosopis velutina* Woot., *Acacia farnesiana* Willd., and *Lysiloma thornberi* Britt. and Rose. All experiments were carried out in the greenhouse during midwinter or late spring, as will be discussed later.

The moisture content of the soil was brought up to 15 per cent, a point well

above the wilting percentage, at the beginning of the experiment.[1] This arbitrary percentage will subsequently be referred to as "favorable." Each pot was weighed daily, and twice the amount of water necessary to bring the moisture to 15 per cent was added. In this way the moisture content was kept fluctuating within narrow limits about the 15 per cent point, except in those pots where the plants were allowed to wilt.

A special type of irrigating apparatus was devised and used in all pots. Four test tubes with holes blown through the bottoms were tied into a bundle, and the four members of each bundle were staggered so that when the apparatus was placed in a vertical position in the soil, water added to each tube was delivered at a different level. One tube delivered water three-fourths of the distance down from the soil surface, a second delivered halfway down, the third one-fourth of the way down, and the fourth allowed water to trickle out just below the soil surface. Daily additions of water to each pot were divided equally among the four tubes. Preliminary trials with *Prosopis* indicated that this irrigation device is more effective in promoting rapid growth than adding all the water to the surface of the soil. No additional water was applied to compensate for the increasing weight of the plants as the experiment progressed.

## Experimentation

The first series of experiments was directed toward the discovery of visible manifestations by the legumes of the attainment of that moisture level at which the leaf blades of mesophytes such as sunflower and wheat wilt permanently.[2]

From one to five leguminous plants were grown in each pot. After these had attained a height of approximately 20 cm., about five grains of wheat were also planted in each of the pots. When the wheat shoots were a few centimeters tall the surfaces of the pots were sealed with wax, and after they were about 15 cm. tall irrigation was suspended. Wheat, which shows wilting promptly and unmistakably, was used only as an indicator of that point in the gradual depletion of soil moisture referred to as the wilting percentage. It was assumed that the root systems of the leguminous and wheat plants growing in the same pot were so intimately associated that the moisture content of the soil would be depleted rather uniformly, and in consequence the water supply available to both species at any one time would be fairly equal. Observations of the degree of branching and the spread of root systems seemed to warrant such an assumption.

By observing the plants closely, it was possible with this technique to discover what definite reactions, if any, would be exhibited by the legumes when the wilting

[1] The field capacity (4, p. 370) of this soil was 19 per cent and the moisture equivalent 17.5 per cent.

[2] Permanent wilting is defined as a degree of wilting so complete that leaves will not regain their normal unwilted appearance after 24 hours in a moist chamber (2).

percentage of the soil was attained. Attention was directed particularly to changes: (*a*) in the appearance of the leaf blades, (*b*) in the normal day and night positions of the leaves, (*c*) in the rate of abscission, and (*d*) in the rate of terminal growth of the shoots.

As controls, an evenly matched series of pots containing legumes and wheat were planted and treated identically, except that the soil moisture was maintained in the vicinity of 15 per cent throughout the experiment rather than allowing the soils to desiccate. This series served as a basis of comparison to evaluate changes in appearance of the experimental plants. Also, these controls were necessary to demonstrate that the phenomena noted in the experimental plants could not be attributed to increasing age, increasing root competition, poorer soil aeration resulting from sealing the pots, or variation in greenhouse environment.

A second series of experiments considered the effect of the attainment of the wilting percentage upon the rate of transpiration in the legumes. Inasmuch as the weighing method was employed to measure transpiration, the technique of interplanting wheat could not be used here, because the water loss of the wheat plants could not be distinguished from that of the legumes rooted in the same soil. As a result of the first series of experiments, however, manifestations of a condition which corresponds to permanent wilting had been observed in the legumes themselves, and these could be used in correlating transpiration behavior with the attainment of the wilting percentage in pots containing only legumes.

In these transpiration experiments the soil surface was sealed with wax after the legumes had attained a height of approximately 20 cm. Each pot was weighed morning and night. After determining the normal diurnal and nocturnal rates of transpiration during a period when soil moisture was maintained at a favorable level, irrigation was suspended. The march of transpiration was then followed with reference to the development of symptoms of wilting such as were discovered in previous experiments.

An atmometer was operated simultaneously on the greenhouse bench throughout this second series of experiments.

## Results

CHANGES IN APPEARANCE OF LEAF BLADES.—The leaf blades of the *Prosopis* plants, which were rooted in the same pot with wheat, exhibited no changes in appearance as the wheat leaves attained the state of permanent wilting and shortly thereafter died. Within a few days after the wilting percentage was attained in the *Acacia* pots, the leaves of this species had gradually assumed a dull, dead appearance and had become so dehydrated as to become brittle. The leaves of *Lysiloma* remained fresh in appearance long after the wilting percentage was attained. Just

before the pinnules of this plant were abscissed, owing to extreme drought, they usually turned bright yellow.

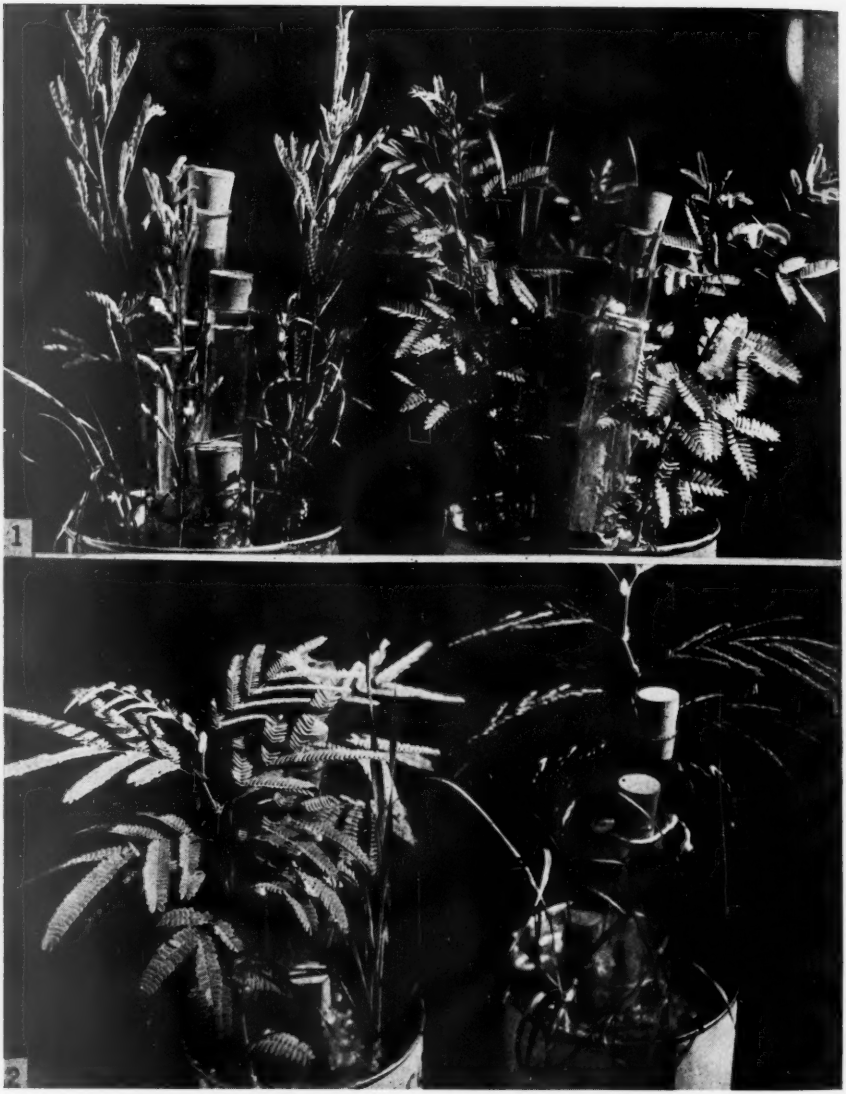
CHANGES IN NORMAL DAY AND NIGHT MOVEMENTS OF LEAVES.—So long as *Prosopis* plants have access to an abundance of soil moisture, the pinnules regularly fold upward at night until paired pinnules are almost in contact with each other above the rachis of the pinna. As drought becomes acute this nyctitrophic response gradually becomes irregular; certain pairs fold while others remain expanded. Furthermore, this irregularity bears no correlation with day or night periods, and there was no consistency in behavior with different parts of the same plant or even with the same leaf.

Normally the pinnules of *Acacia* fold upward together at night, and at the same time the pinnae and petioles bend upward, giving the shoot a fastigiate appearance. When the wilting percentage is attained the leaves assume this nocturnal attitude and maintain it thereafter. The position of pinnae and pinnules in the daytime (fig. 1) is therefore an excellent indicator of whether or not there is growth water in the soil, that is, moisture in excess of the wilting percentages.

Under favorable moisture conditions the pinnules of *Lysiloma* also fold upward together at night and open widely at dawn. If insolation is not intense the leaves of this species remain expanded throughout the day; if insolation is intense, the nocturnal position is resumed a few hours after dawn, even though soil moisture conditions are favorable. Whether or not the wilting percentage has been attained can be determined by observing the plants after sunrise but before the sun is very high, since those plants which are rooted in soil which has reached the wilting percentage do not open their leaves during this period (fig. 2).

CHANGES IN GROWTH RATE.—When the soil moisture became so depleted that wheat wilted permanently, elongation of the shoots ceased abruptly in all three species. In *Prosopis* young leaves continued to expand somewhat after this point was reached, even though leaflets at the bottom of the shoot were abscissing rapidly, owing to drought. The behavior of this species is different from that of maize plants as studied by DAVIS (3). The growth rate of maize decreases with each reduction of the soil moisture content below the capillary capacity and stops when the moisture content approaches a level of about 3 per cent above the wilting percentage.

CHANGES IN RATE OF ABSCISSION.—Daily abscission records of the control series (table 1) showed that there is a slow continual shedding of pinnules in vigorously growing plants of all three species of legumes. In such plants the rate of development of new leaves at the shoot apex far exceeds the rate of abscission at the lower extremity, so that the total leaf surface increases steadily. When the wilting percentage is attained, however, the production of new pinnules ceases and the shed-




FIGS. 1, 2.—Fig. 1, at left, wilted plants of *Acacia constricta* and wheat; at right, plants of same species in unwilted condition. Fig. 2, at right, wilted plants of *Lysiloma thornberi* and wheat; at left, unwilted plants of same species. Photograph made in early morning; a few hours later the pinnules on the unwilted plants of *Lysiloma* also folded together for the day.

ding of old ones increases sharply (table 1), so that the total blade surface is rapidly reduced. The method of recording abscission as the total number of leaflets dropped per day does not give as striking figures as would have been obtained had it been feasible to express abscission as a percentage of the total pinnules on the

TABLE 1

RECORD OF PINNULES DROPPED ON CONSECUTIVE DAYS BY EXPERIMENTAL AND CONTROL PLANTS. POINTS AT WHICH WHEAT WILTED PERMANENTLY INDICATED BY HORIZONTAL LINES. IRRIGATION IN THESE SERIES HAD BEEN SUSPENDED SEVERAL DAYS PREVIOUSLY. CONTROL SERIES IN EACH CASE CONSISTED OF PLANTS EVENLY MATCHED WITH EXPERIMENTAL SERIES FOR VIGOR AND SIZE

| Days | Total pinnules dropped | | | | | |
|---|---|---|---|---|---|---|
| | Prosopis | | Acacia | | Lysiloma | |
| | Seven experimental plants | Seven control plants | Fourteen experimental plants | Fourteen control plants | Four experimental plants | Four control plants |
| 1 | 12 | 1 | 2 | 0 | 5 | 5 |
| 2 | 4 | 1 | 1 | 6 | 6 | 4 |
| 3 | 9 | 0 | 2 | 6 | 5 | 7 |
| 4 | 6 | 1 | 0 | 28 | 9 | 15 |
| 5 | 4 | 0 | 19 | 2 | 12 | 7 |
| 6 | 9 | 4 | 35 | 27 | 16 | 34 |
| 7 | 19 | 3 | 8 | 17 | 0 | 1 |
| 8 | 43 | 3 | 78 | 34 | 11 | 8 |
| 9 | 44 | 4 | 176 | 11 | 6 | 11 |
| 10 | 24 | 7 | 188 | 30 | 31 | 118 |
| 11 | 25 | 4 | 386 | 18 | 466 | 10 |
| 12 | 43 | 9 | 229 | 11 | 80 | 9 |
| 13 | 39 | 6 | 221 | 8 | 82 | 11 |
| 14 | 69 | 7 | 178 | 32 | 56 | 15 |
| 15 | 61 | 3 | 88 | 2 | 46 | 4 |
| 16 | 65 | 2 | 418 | 41 | 49 | 17 |
| 17 | 17 | 5 | 818 | 33 | 56 | 18 |
| 18 | 67 | 13 | 154 | 31 | 52 | 10 |
| 19 | ..... | ..... | 1,179 | 15 | 39 | 6 |
| 20 | ..... | ..... | 1,352 | 10 | 289 | 8 |
| 21 | ..... | ..... | 1,182 | 5 | 215 | 8 |
| 22 | ..... | ..... | 1,259 | 8 | 103 | 15 |
| 23 | ..... | ..... | ..... | ..... | 79 | 42 |
| 24 | ..... | ..... | ..... | ..... | 29 | 6 |

plant, for with cessation of growth, abscission resulted in progressive elimination of pinnules. Even so, the sudden increase in number of pinnules lost per plant was maintained as long as the plants were observed after the wilting percentage had been attained. The irregularities which appear in the records of abscission rate were occasioned when an entire pinna or leaf dropped, carrying a number of pinnules with it.

EFFECT OF ATTAINMENT OF WILTING PERCENTAGE UPON TRANSPIRATION RATE.—Transpiration experiments with *Prosopis* were carried out in December and January, during which season the insolation at Moscow, Idaho, was very feeble and the photoperiod much shorter than that obtaining in the natural range of this species. In an attempt to compensate for such abnormal illumination, a 700-watt lamp, mounted under a reflector, was suspended 1 meter above the greenhouse bench upon which these plants were growing. This supplemental illumination was

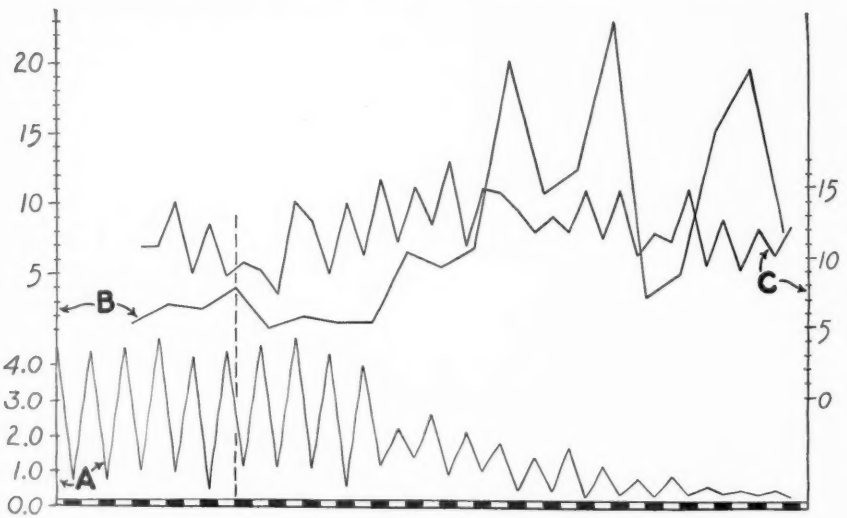



FIG. 3.—March of transpiration, abscission, and evaporation during experiments with *Prosopis: A*, transpiration in grams per plant; *B*, average number of leaflets lost per plant; *C*, atmometric evaporation in cubic centimeters. Alternation of night and day periods indicated at bottom of graph by alternating black and white lines, respectively. Point at which the last irrigation water was supplied is indicated by broken vertical line.

employed daily from 7:30 A.M. to 7:30 P.M., at which times the morning and evening weights of the pots were determined.

In addition to measurements of transpiration and evaporation, a record was kept of the rate of abscission, which in the preceding series of experiments proved a good indicator of the attainment of the wilting percentage in all three species of legumes.

Under conditions of favorable soil moisture and rather uniform illumination, the volume of water lost by *Prosopis* plants during the day was about five times as great as the nocturnal water loss (table 1; fig. 3). After observing this normal rhythm for 5 days, irrigation was suspended. The amount of moisture in the soil

at this time (approximately 15 per cent) was apparently sufficient to allow normal functioning of the plants for about 5 more days, at the end of which the rate of abscission suddenly increased and remained at a high level, and concurrently the rate of diurnal transpiration decreased sharply (fig. 3). The significance of the concomitance of these two observations is strengthened by the fact that there was no sharp decrease in the evaporative power of the air or changes in illumination which could account for the sudden change in transpiration rate. The results of this experiment seem to warrant the conclusion that the normal rate of transpiration in *Prosopis* continues during progressive desiccation until the wilting percentage is attained, at which time the water loss is abruptly reduced. These results are in accord with those of VEIHMEYER (**5**), who worked with young prune trees.

Under the range of evaporative intensities obtained in the greenhouse (fig. 3), transpiration appeared not to be governed by the evaporative power of the air as determined by an atmometer. In fact, transpiration was usually lowest when the evaporative rate was relatively high. Apparently transpiration was closely governed by the amount of illumination. On the other hand, in the absence of wind currents and with fairly uniform temperature, the evaporative rate depended chiefly upon relative humidity, which was usually highest during the day because the greenhouse was sprinkled in the morning. It would have been desirable in connection with these observations of transpiration to know the daily trend of stomatal movement in the legumes, but the pinnules were so small and delicate that none of the methods of studying stomatal apertures known to the writers proved successful.

Another notable feature of the transpiration behavior of *Prosopis* is that the nocturnal rate of water loss appears to be less dependent upon the existence of growth water than does the diurnal rate, although under prolonged drought even nocturnal transpiration is strongly reduced (fig. 3). This may be explained by the fact that at the wilting percentage the plant continues to absorb water, although at a very slow rate, and this quantity goes farther toward meeting the demands of the feeble nocturnal transpiration than of the more vigorous diurnal transpiration.

Experiments with *Acacia* and *Lysiloma* were carried out in May and June, when, except for an occasional cloudy day, insolation was of sufficient intensity and duration so that no supplemental illumination was considered desirable. The evaporation rate at this season varied directly with insolation and resultant temperature fluctuations within the greenhouse, the effect of the latter being so great that all other factors controlling evaporation were subordinated.

The transpiration rate of these two species likewise varied directly with the strength of insolation so long as the soil contained growth water. At this season transpiration was so vigorous that growth water was exhausted within about a day after progressive desiccation started. As in *Prosopis*, plants allowed to dry out ex-

hibited an abrupt decrease in transpiration, accompanied by a rise in the curve of abscission, indicating that the attainment of the wilting percentage is marked by sudden reduction in transpiration. These phenomena were concomitant with the anticipated behavior of pinnules, and in consequence it may be concluded that the results were essentially identical with all three species of legumes.

## Summary

1. The wilting percentage of the soil has essentially the same significance in the water relations of *Prosopis velutina*, *Acacia farnesiana*, and *Lysiloma thornberi* as in wheat.

2. In contrast with wheat, the attainment of the wilting percentage in the legumes is not marked by a wilting of the leaf blades. However, the attainment of the wilting point brings about these changes in the legumes: (*a*) an essentially concomitant increase in the rate of abscission, (*b*) deviation from the normal diurnal positions of the pinnules, (*c*) cessation of shoot elongation, and (*d*) sharp decrease in the transpiration rate.

3. Apparently the growth and transpiration rates of the legumes continue at approximately normal levels as long as the soil contains any water in excess of the wilting percentage.

4. As long as the roots of the legumes have access to growth water, their transpiration rates appear to be closely related to the strength of illumination and to be unrelated to variations in the evaporative power of the air, so long as the latter is not likewise controlled principally by insolation.

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## LITERATURE CITED

1. ALWAY, F. J., Studies in the relation of the non-available water of the soil to the hygroscopic coefficient. Nebraska Agr. Exp. Sta. Bull. 3. 1913.
2. BRIGGS, L. J., and SHANTZ, H. L., The wilting coefficient and its indirect determination. BOT. GAZ. **53**:20–37. 1912.
3. DAVIS, C. H., Absorption of soil moisture by maize roots. BOT. GAZ. **101**:791–805. 1940.
4. LOOMIS, W. E., and SHULL, C. A., Methods in plant physiology. New York. 1937.
5. VEIHMEYER, F. J., Some factors affecting the irrigation requirements of deciduous orchards. Hilgardia **2**:125–284. 1927.

# PECULIARITIES OF THE INFLORESCENCE IN THE EUPHORBIACEAE

LEON CROIZAT

(WITH FOURTEEN FIGURES)

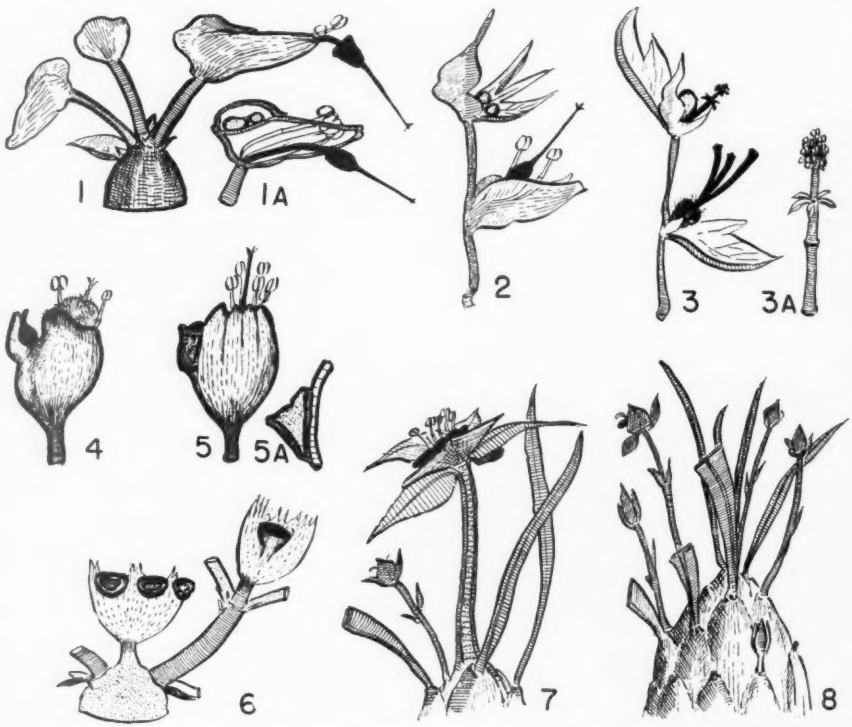
The cyathium of the Euphorbiaceae Euphorbieae is a structure unmatched by other inflorescences in the spermatophytes. It is the purpose of this paper to discuss certain of its aspects, concluding with a cursory review of the epicarp of *Ptychopyxis*, a genus which belongs to the same family if not to the same tribe. In a later paper attention will be given to the inflorescence of certain Celastraceae which suggests considerations of a general nature on the significance of flower-bearing axes.

## I. Cyathium of Pedilanthus

The cyathium of *Pedilanthus* consists of two chambers (fig. 1*A*), one superior and smaller, containing a variable number of glands, the other inferior and larger, mostly tubular in its outline, holding the "ovary," that is, the ♀ flower and numerous stamens which in reality are monandrous ♂ flowers. This "flower" has been interpreted and described by students of the Euphorbieae (BOISSIER in DC. Prodr. **15**(**2**):4. 1862; MILLSPAUGH in Field Mus. Publ. Bot. Ser. **2**:353. 1913) in a manner which is hardly satisfactory. For instance, BOISSIER concludes that the upper lip is merely an external cucullate appendage becoming saccate at its base, including the glands and having no communication with the "tube" surrounding the sexual organs.

To elucidate the features of the cyathium of *Pedilanthus* which have proved embarrassing to BOISSIER and MILLSPAUGH, it is necessary only to tilt this peculiar inflorescence until the gynophore stands almost vertical. This done, it is necessary to suppose that the upper chamber is separated from the lower by a slight elongation of the pedicel. The diagram which figures the outcome of these assumptions (fig. 2) is strikingly reminiscent of the two-storied disposition characteristic for the inflorescence of another euphorbiaceous genus, *Dalechampia* (fig. 3). The floral structures of *Pedilanthus* and *Dalechampia* differ basically only as follows: (*a*) in *Pedilanthus* the upper chamber contains a variable number of glands but no active sexual organs; in *Dalechampia* the upper chamber contains active ♂ flowers in addition to more or less evolute glands; (*b*) in *Pedilanthus* the lower chamber contains one ♀ flower and numerous ♂ flowers; in *Dalechampia* the lower chamber is occupied by three ♀ flowers only. On this basis, if the upper chamber of the inflorescence of *Dalechampia* is deprived of its ♂ flowers and these

are placed around the central ♀ flower of the lower chamber (the lateral ones being suppressed), the cyathium of *Pedilanthus* is seen to appear. The changes hypothetized here to effect a passage from the inflorescence of one to that of the




FIGS. 1–8.—Fig. 1, inflorescence of *Pedilanthus tithymaloides; A*, section through cyathium showing upper chamber with two glands, lower with ♀ flower (ovary) and monandrous flowers (stamens). Fig. 2, diagram illustrating the presumed manner of formation of cyathium of *Pedilanthus*, the upper chamber being produced above the lower in the arrangement characteristic of the inflorescence of *Dalechampia*. Fig. 3, inflorescence of *Dalechampia; A*, ♂ flower. Fig. 4, cyathium of *Pedilanthus tehuacanus*. Fig. 5, same of *P. linearifolius; A*, section through gland. Fig. 6, diagram illustrating euphorbioid cyathium (with several glands) at apex of main stem of *Euphorbia geniculata*, followed above by pedilanthoid cyathium (with one gland) in fork of first dichotomy of umbellaster. Fig. 7, *E. clava:* apex of main stem bearing falsely apical cyathium set upon extension of podaria of main axis. Fig. 8, normal apex of main stem of *E. clava*.

other genus are consistent with the morphology and the sexual expression of the Euphorbiaceae, a family which frequently yields monoecious, dioecious, or polygamous inflorescences in the same genus and even in the same species.

The theory that the appendix of the cyathium of *Pedilanthus* is homologous with the upper bract that incloses the androecium of *Dalechampia*, and that the saccate chamber closed above by this appendix is actually a sterilized androecium but potentially a 1- or 2-sexual inflorescence, invites the objection that the hypothetic elongation of the pedicel above and beyond the lower chamber (fig. 2) is not to be taken for granted. Proof should be given that the stelar continuation of the pedicel reaches the upper chamber of the inflorescence. In view of this objection it must be admitted that the matter does not seem to have received as yet the attention of a specialized morphologist. However, a careful student of the vascular anatomy of the cyathium of *Euphorbia* (HABER in Ann. Bot. **39**:704. 1925) has concluded that in this inflorescence the glands are "a pair of modified secondary branches of a lateral inflorescence"; that is, the glands are primarily caulinar bodies, unlike appendages of foliar origin. Since the glands of *Euphorbia* are often set upon the rim of the involucre in a manner hardly suggestive of stelar bodies, while those of *Pedilanthus* are often found at the bottom of the cavity of the upper chamber, it is not imprudent to assume, until proof to the contrary is given, that the supply of these glands is a branch off that of the pedicel of the inflorescence. Such an assumption has in its favor broad phylogenetic and morphological considerations, not less than the fact that the glands of *Euphorbia* and *Pedilanthus* are admittedly homologous.

Two modifications of the inflorescence of *Pedilanthus* are worthy of notice. In *P. tehuacanus* the tube is sharply truncated (fig. 4), the opening being almost vertical. The appendix is deeply cleft, and, so far as known, no glands are present in the upper chamber. The ovary is rounded as it usually is in *Euphorbia*, not elongate as in typical representatives of *Pedilanthus* (for instance, *P. tithymaloides*). In *P. linearifolius* the upper chamber is exceedingly reduced, appearing as a glandular saccate fold on the side of the vertical, baglike involucre (fig. 5). The tendency inherent in the cyathium of *P. tehuacanus* to depart from zygomorphism with the concomitant reduction of the upper chamber suggests that a further evolution in this type of cyathium may yield a vertical, exappendiculate and eglandular perianth, such as exists in the ♂ cyathium of *E. plagiantha*. This suggests that a shift in the position of the floral axes may have far-reaching consequences upon the nature of specialized reduced inflorescences.

The cyathium of *P. linearifolius*, a species which some treat as *Cubanthus linearifolius*, does not differ in its essential characters from the cyathium of *Euphorbia* (*Poinsettia*) *pulcherrima*. These two species agree closely in floral and vegetative characters and to all appearances are consanguineous. It is possible that the solitary nectary of the cyathium of *E. pulcherrima* is not absolutely homologous with the saccate lateral appendage of that of *P. linearifolius*, and that the former is a much more reduced and specialized structure than the latter. Both

these "glands" have the function of hydatodes, however, which sets them physiologically on a par; it should not be surprising that they differ in morphological details, because relevant differences separate the various kinds of nectaries and glands prevalent in *Euphorbia*. The glands of *E. esula*, *E. jacquiniiflora*, and *E. tridentata* are most unlike, but differences of the kind are correctly interpreted as indicating little that has absolute value in a phylogenetic study. To judge from a sum of characters, *P. linearifolius* and *E. pulcherrima* are the links connecting *Pedilanthus* and more distantly *Dalechampia* with tne aggregate of species commonly treated by taxonomists as *Euphorbia*. The phylogenetic significance of the so-called section Poinsettia of *Euphorbia* is certainly greater than most taxonomists appear to believe, as it is through this section that the prevailingly actinomorphic cyathium of *Euphorbia* can be connected in phylogeny with the zygomorphic inflorescence of *Pedilanthus*, and—more remotely—with the ancestral inflorescence common to all the Euphorbieae. In this connection it is interesting that the species of section Poinsettia are closely related with African and Australian forms in the vicinity of *E. crotonoides* and *E. eremophila*, forming an aggregate (in the broad sense) that enjoys practically a pandemic range.

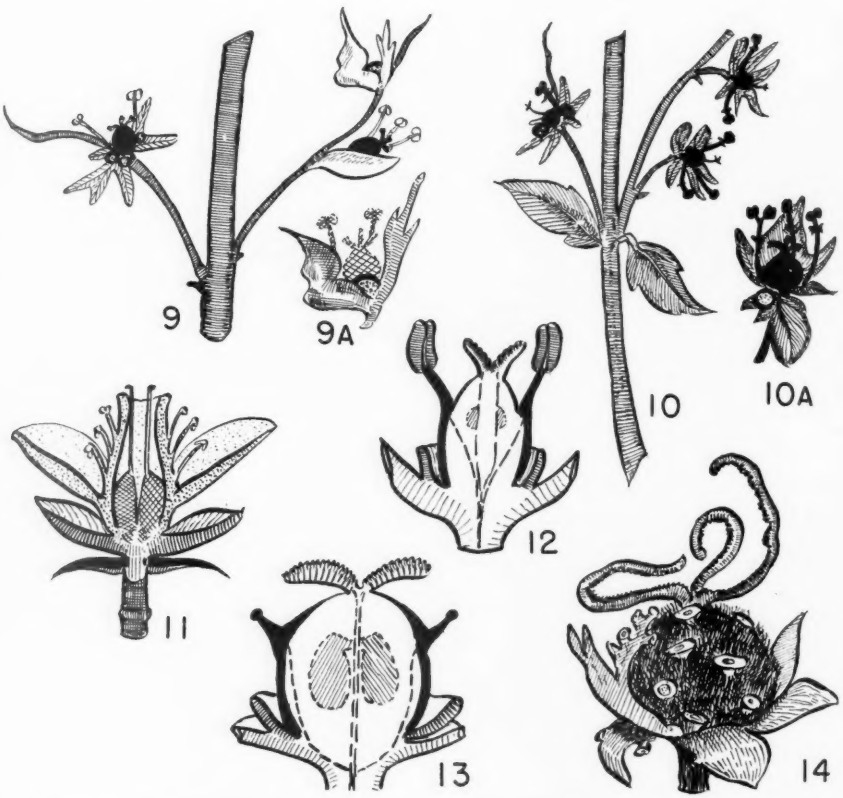
In view of the position occupied by the species of section Poinsettia among the Euphorbieae, it is not surprising to find in these species dimorphic cyathia of considerable interest. In *E. geniculata*, for instance, the primary main stem often ends with a cyathium that may have eight to ten glands; next above, at the first dichotomy of the umbellaster, cyathia occur which are typical of the section and bear only one gland, being the same as those of *E. pulcherrima*. This peculiar arrangement (fig. 6) suggests that in the species of this section a progressive specialization takes place at different levels on the florigerous axes, the same species having first a euphorbioid cyathium (with more than four glands), then poinsettoid cyathia (with only one gland). Such a specialization, as has been noticed, also occurs in *Pedilanthus* (fig. 2), in which the lower chamber is sexually complete and active, the upper sterile, and occupied only by relic bodies of the nature of glands. Moreover, the first cyathium to appear in species of *Euphorbia* outside of section Poinsettia is often unlike the cyathia that follow it. This first cyathium may be unisexual, have nearly free glands and lobes, and especially eight to ten glands and lobes instead of the normal number of four to five. In one species, *E. capitulata*, no umbellasters form, each fertile shoot being terminated by a single cyathium. This cyathium has a status and position closely corresponding to those of the first cyathium of other species of *Euphorbia*. It arises at the origin of the umbellaster, and, like it, has usually eight to ten lobes and glands, a peculiarity which SCHMIDT has used to erect a monotype, *Diplocyathium*. Apparently a cyathium with four to five lobes and glands is not necessarily characteristic of the species of *Euphorbia*, and the cyathia of this genus and of the Euphorbieae in general tend toward dimorphism.

In her study of the cyathium of *Euphorbia*, HABER has concluded (Ann. Bot. 39:694. 1925) that the articulation of the monandrous pseudo-stamen of this peculiar inflorescence is "the receptacle upon which the flower is situated, and in function is similar to that of other receptacles." Since the receptacle may be understood as a region in which earlier growth comes to a stop and new growth begins, it is logical to suppose that a flower that is apparently terminal past an articulation is not necessarily apical. This flower may arise, in fact, from a meristem that is lateral upon the receptacle, occupying an apparent terminal position on the axis on account of the failure of the true apical meristem to develop a flower. An articulation, consequently, may be analogous if not homologous with the scar that marks the insertion of a scion upon the stock to which it has been grafted, and it is not surprising that it should be anatomically complex (LECOMTE in Mus. Nat. Hist. Paris, Nouv. Arch., 5 sér. 2:121–242. 1910). In theory at least, an articulation does not require that the axis come to an abrupt end, establishing a sharp horizontal receptacle. It may form when a lateral meristem acquires preponderant growth, as when, for instance, a lateral bud of *Tilia* or *Ulmus* becomes "terminal" on the shoot on account of the dying-out of the apex of the branchlet upon which this bud is borne. The formation of an articulation may consequently be regarded as the result of a theoretical reversal in the normal ratio of development between the bud gap and the stele, the stele being ultimately disposed of by too continuous a proliferation of lateral buds. In the physiological sense, this happens particularly when dormant structures gain the upper hand against active ones at the end of a length or time of growth. There does not seem to be a sharp line to separate such true articulation, as that of the monandrous flower of *Euphorbia*, described by HABER, from the abscission layer and scar which end a branchlet of *Ulmus* or *Tilia* next above a falsely terminal bud.

The cyathium of *Euphorbia* is apparently terminal, and its pedicel does not exhibit a manifest articulation. It is very likely, however, that this cyathium is not truly apical. *Euphorbia clava*, a succulent which is often cultivated as an ornamental, has main axes which normally do not bear apical cyathia (fig. 8), these being carried upon lateral specialized florigerous axes. In some cases, however, the main stems taper to a peduncle-like end (fig. 7), crowned by a cyathium. Of course this cyathium is not truly apical, because it rests upon a structure of spirally ascending podaria (=decurrent succulent petioles) and is in reality an intercalary inflorescence (PARKIN in Jour. Linn. Soc. Bot. 42:556–558. 1914). What seldom happens in *E. clava* normally takes place in *E. caput-medusae*, in which all cyathia are borne upon peduncles of spirally ascending podaria. In a species in the vicinity of *E. clava* (probably *E. loricata*) I have found by dissection that the bottom of the involucre actually slants upon the supporting axis, showing that the cyathium is not really apical. Thus evidence is forthcoming that the

cyathia of the Euphorbieae are not necessarily and truly apical, despite their appearing in a terminal position upon the axes.

Once it is realized that these peculiar inflorescences are essentially intercalary, that is, that they become falsely terminal by the extinction of the axis which bears




FIGS. 9–14.—Fig. 9, diagram illustrating components of cyathium of *Euphorbia geniculata; A*, outline of gland occupying place of sexual organs in upper chamber (= upper chamber of cyathium of *Pedilanthus;* see fig. 2). Fig. 10, illustrating presumed ancestral inflorescence of Euphorbiaceae Euphorbieae; *A*, detail of glomerule. Fig. 11, *Pavonia hastata:* diagram of flower in longisection. Fig. 12, *Cleistanthus decurrens:* diagram of ♂ flower with pistillode. Fig. 13, showing possible evolution of the ♂ flower of *C. decurrens* by return to functionality of ♀ organs and persistency upon the ovary of sterilized stamens (= staminodes or processes of glandular nature). Fig. 14, flower of *Ptychopyxis thwaitesii* (*Podadenia thwaitesii*) showing presence of actual glands on epicarp.

them in a lateral position, it is not difficult to reconstruct the hypothetical ancestral inflorescence which gave origin to any and all forms of cyathium, that of *Pedilanthus* together with that of *Poinsettia* and *Euphorbia*. The dimorphic

cyathia of *E. geniculata* (fig. 6), for instance, may be homologized to three lateral inflorescences (fig. 9). Of these three, one yields an euphorbioid cyathium by the simple process of fusion of glands and bracts (lobes), four to ten glands becoming adnate and alternate with as many bracts. On the contrary, the pedilanthoid cyathium with one gland is formed by the specialization and subsequent fusion of two inflorescences in a manner characteristic of *Pedilanthus* (figs. 1, 2). In brief, the dimorphism observed in the cyathium of the Euphorbieae in general is the result of the specialization of the inflorescences ancestral to the cyathium, these inflorescences becoming cyathia either as single units or by fusion of two units, it being probable that the euphorbioid cyathium with many glands arises from one inflorescence and the pedilanthoid cyathium with no apparent glands or one gland from the fusion of two inflorescences. The fundamental units in all these inflorescences are the following: (*a*) a central ♀ flower with a much reduced calyculus; (*b*) a variable number of ♂ flowers, with or without a calyculus, bearing one to several stamens; (*c*) glands representing abortive ♂ flowers; (*d*) a variable number of appendages. On this basis the ancestral inflorescence of the Euphorbieae and Dalechampieae, and possibly of the Pereae, might have resembled the inflorescence shown in figure 10. Such an inflorescence is aptly described (BAILLON in Adansonia **6**:354. 1866) as being composed of "Cymes pluripares à fleur femelle centrale," and some of its earliest stages may not have been altogether different from the floral arrangement of *Longetia buxoides* as defined by BAILLON. All these inflorescences relate back to the capitula of the Tiliaceae and to the cymes of the Malvaceae, modified by sexual specialization and by extensive fusion among partial inflorescences and single flowers. Thus the phylogeny of the inflorescences of the Euphorbieae confirms what is known on the strength of other evidence, that the Euphorbiaceae and the Sterculiaceae are closely related, both families arising from the malvoid ancestral group as a case of sexual specialization.

## II. Epicarp of Ptychopyxis

The corolla of malvaceous flowers is described in most textbooks as dialipetalous. This is erroneous, as the corolla in these flowers is intimately connate with the staminal column, the petals and the androecium falling together, calyptrawise, after anthesis. Such flowers in longitudinal section have an outline similar to that shown in figure 11.

*Pavonia hastata*, a species of the Malvaceae, is known to turn "cleistogamous" at certain times of the year (GOEBEL in Organ. Pflanzen. 3 ed. **3**:1971 [fig. 2035]. 1933, CROIZAT in Darwiniana **5**:419. 1941). This "cleistogamy" actually consists of a reduction of the corolla and staminal column which, instead of evolving in full blossom, fail to develop and remain inclosed within the barely opened or even unopened calyx. That this is hardly a case of true cleistogamy need not be emphasized. What actually takes place in *Pavonia* is this: in summer the corolla and

the staminal column develop in full, but for some reason or other the gynoecium remains sterile and no seeds are produced; in winter, on the contrary, the corolla and the staminal column become abortive but the pollen and the ovules are active, so that normal seeds capable of germinating are produced. The background of this differential growth would seem to be metabolic because it is connected with changes in average temperature. The tentative conclusion may be advanced that *P. hastata*—and with it other Malvaceae such as *Sida* spp.—tends toward a mode of sexual expression which involves a departure from the usual bisexual arrangement of the family. This tendency calls into play apetaly and unisexuality.

A malvoid entity which becomes completely apetalous and unisexual is turned, *ipso facto*, into a sterculiaceous and euphorbiaceous plant because the Sterculiaceae in part and the Euphorbiaceae as a whole differ essentially from the Malvaceae in being apetalous and unisexual. No sharp morphological line can be drawn to separate the Malvaceae, Tiliaceae, Sterculiaceae, and Euphorbiaceae, for they differ in tendencies more than in characters. Thus *Pavonia* and *Sida* illustrate the beginning of a type of floral evolution which eventually ends by establishing the cyathium of *Euphorbia*. The first step in this evolution is probably taken when the corolla and the staminal column become intimately connate, for it is then that a type of flower which is found in the Theaceae and, farther still, in the Magnoliaceae, becomes actually malvoid. As a matter of fact, the entire evolution may be tentatively outlined as follows: (*a*) the syncarpic, conelike gynoecium becomes reduced, only the basal row of carpels surviving either dialicarpically or syncarpically; (*b*) the stamens fuse together and with the corolla; (*c*) either the corolla or the staminal column evolves, ultimately leading to unisexuality; (*d*) unisexuality being attained, the flower undergoes further reduction in detail. All these steps merely postulate concentration and specialization of the various floral organs, that is, an essential progression in reduction. It is not surprising that in the families in which unisexuality and reduction prevail, such as the Sterculiaceae and the Euphorbiaceae, nectaries are frequently found together with pistillodes, these organs representing surviving but degenerate parts of the androecium or of the gynoecium.

In *Pavonia hastata* the gynoecium may become sterile, either anatomically or physiologically, this being a point upon which no information seems as yet available, and the androecium (corolla and staminal column) may become reduced, simulating cleistogamy and actually presenting a form of apetaly. In numerous Euphorbiaceae the ♀ organs are abortive and reduced to pistillodes or "glands," while the ♂ organs are fully developed, the phylogenetic link between the Malvaceae and the Euphorbiaceae being furnished by the Sterculiaceae. An especially revealing example of flower in which a pistillode is surrounded by stamens is that of *Cleistanthus decurrens* (fig. 12), a euphorbiaceous plant.

In the light of the tendencies prevalent in the malvoid-euphorbioid phylum it is permissible to suppose that the pistillode of *C. decurrens* might again become sexually active and anatomically perfect in connection with the process of ovule- and seed-bearing, the stamens being turned into staminodes, that is, into sterilized stamens or glandular bodies. As the result of this supposition a structure is visualized of the kind shown in figure 13, in which the staminode or its equivalent, a gland, persists upon the ovary and the fruit as a process or an appendage, the androecium (staminal column and corolla) becoming part of the epicarp.

The hypothesis that the processes upon the capsule of certain Euphorbiaceae are actually relics from an abortive androecium may not be true in every case, but it certainly explains manifestations which are otherwise difficult to account for. In certain species of *Codiaeum*, for instance, the epicarp is beset with glands that do not differ in the slightest from floral nectaries. Such nectaries are numerous upon the ovary and fruit of *Ptychopyxis thwaitesii* (fig. 14) and are in no wise distinct from the staminodes and glands normally situated next to the calyx. It is well known, moreover, that in *Ricinus* the prickles upon the capsule have a vasculation of their own which is not suggestive of epidermal processes as such. In conclusion: some evidence is forthcoming that in the malvoid flower and its derivative structures, the carpels as well as the stamens may become intimately connate and adnate with the corolla, which is fully in harmony with the tendency toward reduction and unisexuality prevailing in the malvoid and euphorbioid phylum. Since it is admitted—on the mere strength of visual evidence—that the abortive gynoecium of the Euphorbiaceae persists as a pistillode (*Cleistanthus*) or as discrete glands (*Tetracoccus*, *Securinega*) in the ♂ flower, it should not be surprising that the androecium may persist as glands or other processes, free from the epicarp (*Croton*) or intimately connate with it (*Ptychopyxis*) in the ♀ flower.

## Summary

1. The cyathia of *Euphorbia* and *Pedilanthus* represent different degrees of adaptation and fusion in a single type of compound lateral inflorescence. Such an inflorescence was probably intercalary in its origin.

2. A tendency toward the reduction of floral parts involving apetaly and asexuality appears in the Malvaceae and becomes dominant in the Sterculiaceae and the Euphorbiaceae.

3. The glands and processes on the epicarp of *Codiaeum*, *Ptychopyxis*, and *Ricinus* are apparently relics from the androecium that became intimately fused with the carpels. Glands of the same nature probably appear also in other families (Sabiaceae).

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# EFFECTS OF PHOTOPERIOD ON SEX EXPRESSION IN AMBROSIA TRIFIDA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 542

LOUIS K. MANN

(WITH FIVE FIGURES)

## Introduction

It is known that photoperiod may exert marked influence upon the sex expression of certain monoecious and dioecious plants (**5**). In *Zea* and *Cannabis*, for example, shortening the photoperiod to less than that to which the plants are usually exposed at the time of flowering causes a "reversal" of the staminate (*Zea*) (**8**) or both staminate and pistillate (*Cannabis*) flowers (**6, 7**). In experiments carried out to determine the critical photoperiod of giant ragweed, *Ambrosia trifida* L., a short-day plant, it was noticed that among plants exposed to relatively short photoperiods (less than 9 hours) there was a reduction in the number of staminate involucres relative to the number of pistillate involucres, and in some cases racemes which were usually staminate contained some pistillate involucres.

The following experiments were designed to determine to what extent sex expression in giant ragweed could be modified by photoperiodic treatment, and, in this respect, to compare the effects of varying both the length of photoperiod and the number of photoinductive cycles to which the plants were exposed.

## Material and methods

Experiments were carried out in the greenhouses at Chicago from June until October, 1941. Seeds were collected locally in the autumn of 1939 and kept in the refrigerator to break dormancy (**2**). The seeds were planted in flats on June 16, and the seedlings were later transplanted to 4-inch pots. The pots were placed on benches under natural daylight with the photoperiod extended until 10:00 P.M. On July 18 the photoperiod was further extended until midnight by Mazda light (200-watt bulbs in porcelain reflectors, kept 1–2 feet above the tips of the plants). Plants were selected for uniformity and treatments were started July 18.

In addition to the long-day bench, photoperiods of 15, 12, and 6 hours were used. The 12- and 15-hour photoperiods were obtained by covering frames on the greenhouse benches with a layer of black sateen cloth at 6:00 P.M. and 9:00 P.M., respectively, and removing the cloths at 6:00 A.M. the next day. Plants on the 6-hour photoperiod were placed in a darkroom between 5:00 and 6:00 P.M. and were returned to the greenhouse between 11:00 and 12:00 A.M. the following day. This

procedure for the longest dark period avoided the high temperatures which develop under black cloth during daylight.

The plants were treated in units consisting of approximately 100 plants; in all, ten of these experimental units (1000 plants) were used. The units were given the following treatments. One unit remained on the long-day bench until the end of

FIG. 1.—*A*, racemes of staminate involucres terminating main axis and lateral branches; pistillate involucres in leafy bracts below. *B*, suppression of long laterals common in greenhouse plants.

the experiment as a control. The remaining nine units were divided into three groups of three units each. The first group of three units was exposed to 15-hour photoperiods, one unit receiving two cycles, one receiving ten cycles, and the third receiving twenty cycles of this photoperiod before being returned to the long-day bench, where they all remained until the end of the experiment. In the second group the units were exposed to two, ten, and twenty cycles of 12-hour photoperiod before being returned to the long-day bench. The third group was similarly exposed to two, ten, and twenty cycles of 6-hour photoperiod and then returned to the long-day bench.

In general there was marked variation, even among plants within a single uniform treatment. In extreme cases, plants bearing fruit and other plants just beginning to differentiate flower primordia were found within a single unit. This variation necessitated the use of the large numbers of plants in each unit.

## Results

In comparing ragweed plants which have received various specific photoperiodic treatments with field plants, it must be recognized that environmental factors

FIG. 2.—*A*, elongation of branch bearing pistillate involucres. *B*, cymose arrangement of pistillate involucres.

other than photoperiod alter the appearance of the plant when it is grown in the greenhouse. Since, in the ragweed, staminate and pistillate flowers are located on different parts of the plant, changes in vigor, height, branching, etc., may affect the relative proportion of pistillate and staminate flowers. Plants in the field have a dominant central axis with well-developed lateral branches which branch again several times. The terminal portion of the primary axis and those of most lateral branches are racemes of involucres of staminate flowers. The sessile or subsessile pistillate involucres are in small cymose clusters, each involucre being subtended by a leafy bract. For the most part these short cymose branches occur on secondary branches, or occasionally on the main axis as supernumerary branches in a

leaf axil immediately below the main branch in that axil. The racemes of staminate involucres and the leafy bracts containing the pistillate involucres are shown in figure 1*A*.

In general, plants grown in the greenhouse vary from field plants in the absence of most or all of the long secondary branches on the main axis (fig. 1*B*). The sec-

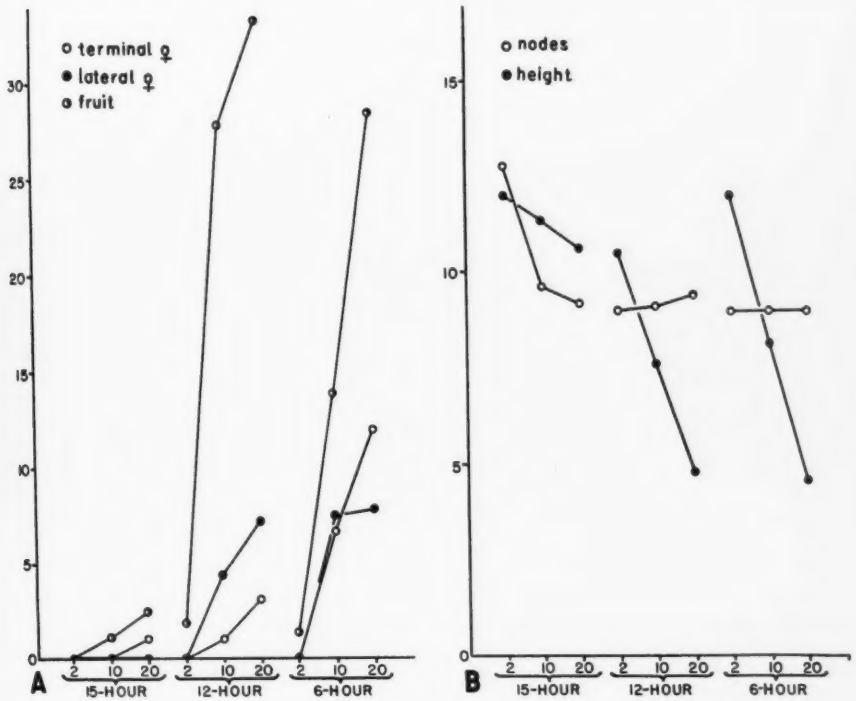


FIG. 3.—*A*, number of mature fruits per plant; number of terminal and lateral staminate racemes per 100 plants in which some or all staminate involucres were replaced by pistillate involucres. *B*, number of nodes and average height in decimeters of plants at maturity.

ondary branches, which are usually rather long, may fail to elongate, the terminal racemes being present but aborted. In other cases the terminal portion of the lateral branch may be replaced altogether by a typical cymose pistillate branch (fig. 2*B*). Occasionally such a branch (fig. 2*B*) may elongate (fig. 2*A*).

In so far as the primary interest in the plants was in the flowering and fruiting structures, most of the data were taken when the plants were mature (fruits ripe and leaves beginning to brown). In those groups which received only two photoinductive cycles, regardless of their length, the maturation was so slow that the

plants were harvested before maturity. Thus the data from these units, with respect to number of mature fruits and height, are not wholly comparable with the other units. The control unit was macroscopically vegetative at the time of harvest, but on dissection most plants were found to be initiating flower primordia. This was probably the result of an irregularity in the electric-power supply which operated the lights. Previous work on ragweed seems to indicate that it remains vegetative under photoperiods of less than 9 hours.

FIG. 4.—*A*, large clusters of pistillate involucres in leaf axil on main axis of plant. *B*, reversion of terminal raceme to vegetative condition.

Rate of maturity and intensity of photoperiodic response are probably best indicated by the height of the plants (fig. 3*B*). Uniformity in number of nodes indicates simultaneous induction of most units, the variation in height being due primarily to the length of the internodes. On the basis of rate of maturity, the unit given twenty 12-hour photoinductive cycles was induced most strongly, this group —on the average—maturing several days before any of the other units.

With respect to yield of fruit (fig. 3*A*), the unit receiving twenty 12-hour photoinductive cycles is again greater than the others. In this group the short cymose branches at the nodes of the main axis were well developed. A cluster of these branches with its many fruits is shown in figure 4*A*. In the units which received

only two photoinductive cycles, these short pistillate branches were poorly developed, usually not more than six fruits at a node.

Although the yield of fruit may be an indication of the balance between pistillate and staminate flowers, it cannot be taken directly as an indication of differentiation of staminate structures instead of pistillate. Although the position of pistil-

FIG. 5.—*A*, *B*, lateral racemes in which staminate involucres have been replaced by pistillate involucres. *C*, tip of terminal raceme with replacement of staminate involucres by pistillate involucres.

late involucres varies, even in field plants, the staminate involucres are confined to terminal racemes, either of the main axis or of the lateral branches. In plants which received short photoperiods, the staminate involucres in these racemes failed to appear, pistillate flowers developing in the position usually occupied by staminate. The development of pistillate involucres on the lateral racemes is shown in figure 5*A*, *B*, while a portion of a terminal raceme with differentiation of pistillate flowers is shown in figure 5*C*. Figure 3*A* shows the increasing incidence of pistillate involucres in racemes with increasing numbers of photoinductive cy-

cles and decreasing lengths of photoperiod. In several plants both terminal and lateral racemes developed pistillate involucres, resulting in completely pistillate plants.

In addition, various gradations between staminate and pistillate structures were found. Reversions to vegetative conditions (fig. 4*B*) were also common under short photoperiods. Variations of this type in other plants have been rather thoroughly described (1). The presence of simple leaves rather than the usual lobed type is not uncommon in plants in the field (3). Percentage of leaves which were simple varied from 18 to 31 under different treatments, the higher percentages being found in the lots which received smaller numbers of photoinductive cycles.

In *Ambrosia elatior*, variations in the inflorescence are found in populations growing in the field. These are similar to those brought about in *A. trifida* by very short photoperiods. JONES (4, 5) has demonstrated that the variations in *A. elatior* are associated with genetic forms. He has found that long photoperiod may modify expression in some strains of *A. elatior*, but that the usual monoecious form which breeds true was stable with respect to environmental changes. JONES found that *A. elatior*, of any genetic form, when subjected to short photoperiod was dwarfed and might fail to produce staminate involucres, but that "such pistillate plants, in a culture which should be monoecious, cannot be regarded as 'sex reversals,' since pistillate flowers have not replaced staminate flowers." In *A. trifida*, on the other hand, length of photoperiod and number of photoinductive cycles have a pronounced effect on sex expression. Lacking experimental information, the uniformity in field plants would suggest that genetic factors are of less importance in *A. trifida* than in *A. elatior*.

## Summary

1. *Ambrosia trifida*, a short-day plant, after starting growth on long day, was subjected to two, ten, and twenty photoinductive cycles at each of three photoperiods—15, 12, and 6 hours. Following this treatment, the plants were returned to a long-day bench to complete growth.

2. Rate of maturity was most rapid and yield of fruit was greatest in the plants exposed to twenty photoinductive cycles at the 12-hour photoperiod.

3. Sex expression as measured by development of pistillate flowers in the position usually occupied by staminate increased with increasing numbers of photoinductive cycles and decreasing lengths of photoperiod, being greatest in the plants exposed to twenty photoinductive cycles at the 6-hour photoperiod.

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## LITERATURE CITED

1. BIDDULPH, O., Histological variations in cosmos in relation to photoperiodism. BOT. GAZ. **97**:139–155. 1935.
2. DAVIS, W. E., Primary dormancy, after-ripening, and the development of secondary dormancy in embryos of *Ambrosia trifida*. Amer. Jour. Bot. **17**:58–76. 1930.
3. DEAM, C. C., Flora of Indiana. Department of Conservation, Division of Forestry, Indianapolis. 1940.
4. JONES, K. L., Studies on *Ambrosia*. I. The inheritance of floral types in the ragweed, *Ambrosia elatior* L. Amer. Mid. Nat. **17**:673–699. 1936.
5. ———, Studies on *Ambrosia*. II. Effect of certain environmental factors on floral development of *Ambrosia elatior*. BOT. GAZ. **98**:296–306. 1936.
6. LOEHWING, W. F., Physiological aspects of sex in angiopserms. Bot. Rev. **4**:581–625. 1938.
7. SCHAFFNER, J. H., The influence of relative length of daylight on the reversal of sex in hemp. Ecology **4**:323–344. 1923.
8. ———, Sex reversal and the experimental production of neutral tassels in *Zea mays*. BOT. GAZ. **90**:279–298. 1930.

# EFFECT OF CHEMICAL TREATMENTS IN PROLONGING DORMANCY OF TUNG BUDS

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(WITH TWO FIGURES)

## Introduction

Several investigators have reported experiments in which the growth of buds was inhibited by treatment with various organic compounds. Among the first to propose a theory to account for the dominance of terminal buds in plants was LOEB (6). He suggested that there might be a substance moving downward from growing apical buds which inhibits growth of the lower buds. This theory is supported by the studies of THIMANN and SKOOG (9). GUTHRIE (2) noted that the potassium salt of alpha naphthalene acetic acid inhibited growth of buds in "non-dormant" potato tubers. Later, GUTHRIE (3) also observed that the vapors of the methyl ester of alpha naphthalene acetic acid produced the same effect. MITCHELL and STEWART (7), studying the growth responses produced by plant hormones, found that alpha naphthalene acetamide and alpha naphthalene acetic acid inhibited development of the terminal buds and expansion of primary leaves when applied to green plants in strong concentrations in the form of emulsion sprays. About the same time LINDNER (5) found that high concentrations of indoleacetic acid and alpha naphthalene acetic acid in lanolin (smeared on cut surfaces) increased the quantity of roots of horseradish but decreased the number of shoots in segments of the root. Recently WINKELPLECK (10) reported delaying the blossoming of excised branches of stone fruits for several days with various derivatives of alpha naphthalene acetic acid.

Whether the work of GARDNER, MARTH, and BATJER (1) and others on the inhibition of preharvest drop of apples by treatment with such compounds as alpha naphthalene acetic acid is a growth inhibition phenomenon is not clear at this time.

One of the most important problems confronting the tung industry today is the injury to the flowers by late spring frosts. During 2 out of the last 5 years, late spring frosts have very seriously reduced the production of tung oil in America.

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It is safe to say that if this hazard could be eliminated, the average production over a term of years could be increased by at least 25 and perhaps 50 per cent. For the last two seasons work has been in progress to test several substances that might prolong dormancy long enough to reduce the likelihood of damage from such frosts. The purpose of this paper is to report the results obtained in prolonging dormancy of tung buds by means of certain organic compounds.

## Investigation

Healthy tung trees were used in an orchard near Floral City, Florida. Two to four representative branches, each having an average of thirty to sixty buds, were

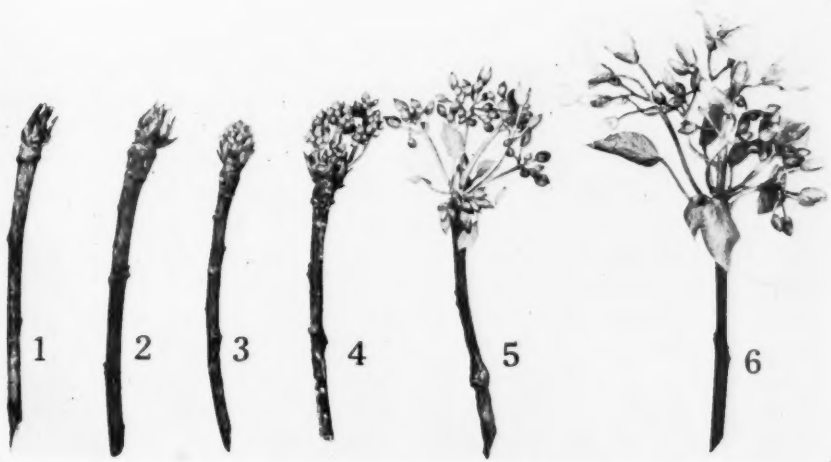


FIG. 1.—Specimens of tung buds illustrating degree of development of the first six classes

selected on each tree. Half of these were treated and the remainder used as controls. This branch unit method was adopted rather than a tree unit method because of the great variability in the blooming habit among individual seedling trees. Each treatment was applied to one branch on each of three different trees.

Three series of treatments were used, beginning February 10, February 25, and March 11, respectively. In each case repeat applications were made at intervals of 2 weeks until March 25. Thus four applications were required for the first series, three for the second, and only two for the series begun on March 11.

Three methods of application were used: (*a*) Direct injection (by means of a hypodermic syringe) of a 0.01 per cent aqueous solution of the potassium salt of alpha naphthalene acetic acid into the bud scale sheath formed around the flower primordia of dormant buds. (*b*) Application to the surface of the dormant buds

and twigs by means of a pressure sprayer, using two different spray formulas. In one case, a 0.01 per cent solution of alpha naphthalene acetic acid was prepared in a mixture containing 4 per cent dioxane and 1 per cent fish-oil soap solution; in the other mixture approximately 0.4 per cent by weight of summer spray oil was substituted for the fish-oil soap. The compounds indole-3-acetic acid and alpha naphthalene acetamide were substituted for the above substance in other spray treatments. In each case the organic substance was dissolved in the dioxane before mixing with the remainder of the ingredients. (*c*) Application of the organic substance in a lanolin emulsion to the surface of dormant buds by means of a small varnish brush. The emulsion was prepared according to the formula of HILDRETH and MITCHELL (**4**). However, 0.3 per cent solutions of alpha naphthalene acetamide and of indole-3-acetic acid were substituted for indole-3-butyric acid.

In order to assay the effectiveness of the treatment in prolonging dormancy, seven classes of buds were established on the basis of the degree of development. The midpoint of each class is typified by the specimens illustrated in figure 1. Class 7 (not illustrated in the figure) represents terminals on which petals have begun to fall. When the buds began to expand counts were made at intervals of 3–7 days to determine the comparative number of buds in each class on treated and control branches.

Of the three methods listed, only the last (*c*) produced any observable effect on dormancy of the buds. Whether the difference in the effectiveness of these methods is due to the difference in concentration of organic substances or to the manner of application has not been established. That the effect of prolonging dormancy was due to the organic substance rather than to the lanolin carrier was shown by field blank tests comparing buds treated with the lanolin carrier alone and untreated buds. No difference could be observed between these two groups. This was further confirmed by laboratory experiments with excised tung branches.

The data presented in table 1 summarize the results obtained with alpha naphthalene acetamide in lanolin emulsion. A similar trend was obtained with indole-3-acetic acid in the same carrier. Similar results were also obtained with excised tung branches brought into the laboratory, treated as outlined in (*c*), then placed in humid chambers with cut ends standing in about 2 inches of water.

The data indicate a pronounced effect of the treatment in delaying bud development. Those treatments in which applications were repeated three or four times were about equally effective in delaying bud development, but when the buds received only two applications, the effectiveness was slightly decreased. It is not yet possible to state whether the number of times the treatment was repeated or the delayed date of initial application was the more important factor in producing the decreased effectiveness of the treatments repeated only twice.

Preliminary experiments by FERNHOLZ and POTTER (**8**) indicate that the im-

TABLE 1

EFFECT OF ALPHA NAPHTHALENE ACETAMIDE IN LANOLIN EMULSIONS IN PROLONGING DORMANCY IN TUNG BUDS; 1941 SEASON

| Date of first application | No. of times treatment repeated | Date of bud count | State of bud development (data as percentages) | | | | | | | | | | | | | | Index* | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Class 1 | | Class 2 | | Class 3 | | Class 4 | | Class 5 | | Class 6 | | Class 7 | | | |
| | | | Treated | Controls | Treated | Controls | Treated | Controls | Treated | Controls | Treated | Controls | Treated | Controls | Treated | Controls | Treated | Controls |
| 2/10 | 4 | 4/4 | 94.2 | 45.6 | 4.3 | 45.6 | 1.5 | 6.6 | 0 | 2.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 107 | 165 |
| 2/10 | 4 | 4/8 | 78.7 | 5.4 | 13.2 | 13.5 | 5.9 | 20.8 | 2.2 | 53.7 | 0.0 | 6.6 | 0.0 | 0.0 | 0.0 | 0.0 | 132 | 343 |
| 2/10 | 4 | 4/11 | 28.4 | 0.4 | 31.4 | 6.3 | 33.6 | 24.2 | 4.4 | 18.8 | 2.2 | 36.8 | 0.0 | 13.5 | 0.0 | 0.0 | 221 | 426 |
| 2/10 | 4 | 4/16 | 14.0 | 0.5 | 3.7 | 0.0 | 2.2 | 0.5 | 63.9 | 15.0 | 6.6 | 7.3 | 1.5 | 58.9 | 8.1 | 17.8 | 382 | 577 |
| 2/10 | 4 | 4/23 | 8.4 | 0.0 | 1.7 | 0.0 | 3.4 | 0.0 | 0.0 | 0.0 | 7.6 | 0.0 | 31.0 | 1.9 | 47.9 | 98.1 | 581 | 698 |
| 2/25 | 3 | 4/4 | 89.7 | 36.3 | 9.3 | 41.4 | 1.0 | 21.1 | 0.0 | 1.2 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 111 | 187 |
| 2/25 | 3 | 4/8 | 82.0 | 5.4 | 6.6 | 13.5 | 10.4 | 20.8 | 1.0 | 53.7 | 0.0 | 6.6 | 0.0 | 0.0 | 0.0 | 0.0 | 130 | 343 |
| 2/25 | 3 | 4/11 | 57.3 | 0.4 | 16.7 | 6.3 | 10.4 | 24.2 | 7.3 | 18.8 | 7.3 | 36.8 | 1.0 | 13.5 | 0.0 | 0.0 | 194 | 426 |
| 2/25 | 3 | 4/16 | 29.8 | 0.5 | 3.2 | 0.0 | 8.5 | 0.5 | 40.4 | 15.0 | 2.1 | 7.3 | 12.8 | 58.9 | 3.2 | 17.8 | 333 | 577 |
| 2/25 | 3 | 4/23 | 24.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 4.3 | 0.0 | 8.5 | 0.0 | 13.8 | 1.9 | 48.9 | 98.1 | 509 | 698 |
| 3/11 | 2 | 4/4 | 61.9 | 25.2 | 28.6 | 36.4 | 7.5 | 38.4 | 2.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 150 | 213 |
| 3/11 | 2 | 4/8 | 42.6 | 0.6 | 10.5 | 3.1 | 20.3 | 13.6 | 26.6 | 75.3 | 0.0 | 7.4 | 0.0 | 0.0 | 0.0 | 0.0 | 231 | 386 |
| 3/11 | 2 | 4/11 | 23.8 | 0.6 | 17.3 | 1.9 | 25.2 | 6.2 | 20.1 | 27.8 | 12.2 | 45.6 | 1.4 | 17.9 | 0.0 | 0.0 | 284 | 470 |
| 3/11 | 2 | 4/16 | 18.9 | 0.0 | 1.4 | 0.0 | 1.4 | 0.0 | 36.3 | 6.9 | 2.1 | 4.4 | 23.8 | 72.5 | 16.1 | 16.2 | 437 | 598 |
| 3/11 | 2 | 4/23 | 8.9 | 0.0 | 0.0 | 0.0 | 3.4 | 0.0 | 0.7 | 0.0 | 6.9 | 0.0 | 21.2 | 0.0 | 58.9 | 100.0 | 596 | 700 |

* Obtained by summing the products of the percentage in each class and the class number; hence the smaller the index figure, the more retarded the bud development.

mature peduncles of tung flower buds are exceedingly susceptible to injury by low temperature. Thus when terminals in the tight cluster stage (class 3) are exposed to a temperature of 28° F., very little injury occurs. When open clusters with the peduncles exposed (classes 4 and 5) are subjected to a temperature of 28° F., all are either injured or killed.

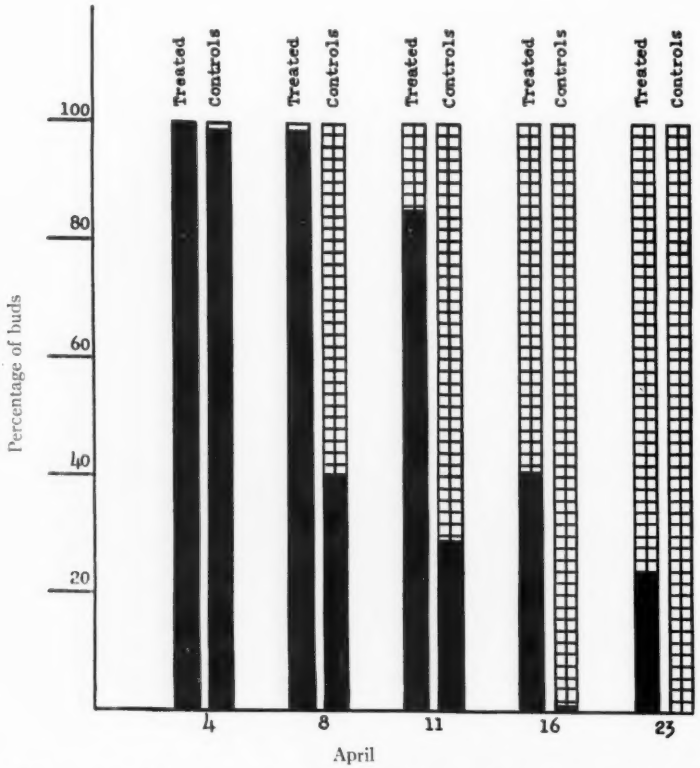


FIG. 2.—Degree of development of tung buds treated three times with alpha naphthalene acetamide as compared with untreated controls. Solid sections of columns represent classes 1, 2, and 3 (buds uninjured by temperature of 28° F.); open sections of columns represent classes 4, 5, 6, and 7 (buds seriously injured or killed by temperature of 28° F.).

These studies suggest that buds in class 4 or above will be damaged seriously by a light to medium frost, whereas it would take a heavy frost seriously to damage buds in classes 1, 2, and 3. In view of these considerations, the data in table 1 are expressed graphically in figure 2 to show the relative frost resistance to treated and control buds at the dates indicated on the graph. The more resistant buds in

classes 1, 2, and 3 were combined into one group and are represented by the solid portions of the bar graphs. The more expanded buds in the remaining less resistant classes are represented by the open portions. It may be noted that there was a period of about one week during which the injury to the treated branches by a frost of about 28° F. would probably have ranged between 0 and 15 per cent, whereas the injury to the untreated branches might have ranged from slight to 70 per cent. During the ensuing 12-day period the treated branches had from 24 to 40 per cent or more of buds not susceptible to injury by a light frost, while all or nearly all the buds on the controls would have been injured.

The data presented here are concerned merely with observations on prolonging dormancy of tung buds by means of certain organic compounds. No practical applications are suggested or recommendations intended. There is a possibility, however, that some practical applications may develop from future studies.

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## LITERATURE CITED

1. Gardner, F. E., Marth, P. C., and Batjer, L. P., Spraying with plant growth substance for control of the pre-harvest drop of apples. Proc. Amer. Soc. Hort. Sci. **37**:415–428. 1940.
2. Guthrie, J. P., Inducing "dormancy" in potato tubers with potassium naphthalene acetate and breaking it with ethylene chlorohydrin. Science **88**:86. 1938.
3. ———, Inhibition of the growth of buds of potato tubers with the vapor of the methyl ester of naphthalene acetic acid. Contr. Boyce Thompson Inst. **10**:325–328. 1939.
4. Hildreth, A. C., and Mitchell, J. W., Spraying is a new method of applying root promoting substances. Florists Review **84**:13. 1939.
5. Lindner, R. C., Effects of indoleacetic and naphthylacetic acids on development of buds and roots in horseradish. Bot. Gaz. **100**:500–527. 1939.
6. Loeb, J., A quantitative method of ascertaining the mechanism of growth and of inhibition of growth of dormant buds. Science **45**:436–439. 1917.
7. Mitchell, J. W., and Stewart, W. S., Comparison of growth responses induced in plants by naphthalene acetamide and naphthalene acetic acid. Bot. Gaz. **101**:410–427. 1939.
8. Fernholz, D. L., and Potter, G. F., Private communication.
9. Thimann, K. V., and Skoog, F., Inhibition of bud development and other growth substance in *Vicia faba*. Proc. Roy. Soc. London B. **114**:317–319. 1934.
10. Winkelpleck, R. L., Blossom delay with hormones. Amer. Fruit Grower **60**:4. 1940.

# EFFECTS OF STORAGE CONDITIONS ON CUT ROSES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 543

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## Introduction

Successful storage of cut flowers should achieve several results: (*a*) The flowers should remain in the desired state of maturity or tightness of flower buds, during storage. (*b*) The flowers should retain their original color and color shadings or an equally satisfactory color, during storage. (*c*) It should be possible to store the flowers for a considerable period of time. (*d*) After removal from storage the flowers should retain their color and turgidity as long as would freshly cut flowers.

The rose, the most important flower in the florist industry, is one of the most difficult to store successfully. Under most conditions the cut buds develop characteristics that render them unsatisfactory when marketed in competition with those freshly cut. Various suggestions have been advanced toward a solution. PERRET (**14**) advocated the use of low temperatures and stressed the importance of the proper relative humidity during storage. HITCHCOCK and ZIMMERMAN (**6**) also demonstrated effects of these factors in relation to specific flowers. They stated that flowers stored at 5° C. for 7 days or longer may be expected to wilt much sooner when removed to a temperature above 20° C. than those freshly cut. NEFF and LOOMIS (**13**) and NEFF (**11, 12**) emphasized the importance of storing flowers with the flower stems out of water. THORNTON (**19**) found that suitable concentrations of carbon dioxide in the atmosphere of the storage container tended to prevent opening of rose buds during the storage period. Those stored in a carbon dioxide-enriched atmosphere lasted longer after removal to ordinary room conditions than similar ones that had been stored in an unenriched atmosphere. THORNTON considered 7 days the approximate limit of effective storage with carbon dioxide, because of petal drop.

The effect of storage conditions on the color of flowers has received considerable study. THORNTON (**20**) found that changes in the color of anthocyanin pigments of rose petals occurred when exposed to high concentrations of carbon dioxide. The bluing of the petals, that is, their tendency to become violet or purplish as they age, was correlated with changes in pH; the pH increased with the use of increased concentrations of carbon dioxide. In the case of Templar roses the loss of original color was correlated with a 0.27 increase in pH.

Change in color shading during storage is difficult to control. To overcome this

major difficulty it may be possible to stabilize the color in roses in such a way that storage conditions will not affect it. This may be accomplished eventually by treating natural coloring substances as dyes and stabilizing them in the petals, in somewhat the same manner that some dyes are stabilized. Most of the natural plant colors are mordant dyes (**10, 15**); that is, they must be adsorbed by a mordant or mordants if the color is to be stabilized. A mordant will adsorb a dye in the presence of a fiber (**22**) or combine with a dye (**16**) in forming insoluble colored compounds. Anthocyanin pigments, which constitute many of the colors of flowers, when mordanted with metallic salts tend to give dyeings fast to light but not to soap and water (**15**). Although theoretically any salt of a heavy metal is capable of acting as a mordant and adsorbing mordant dyes (**10**), in actual practice some salts are superior to others in this respect. Acid mordants include tannins, fatty acids, albumin, hydrous silica, arsenic acid, phosphoric acid, and various others. Metallic mordants are mainly hydrous oxides of the heavy metals, the most important of which are those of aluminum, chromium, copper, iron, and tin (**22**).

The color of a dye may be changed, depending on the mordant used. Alizarine red, for example, when dyed on a chrome mordant results in purplish red, on aluminum in bright red, on tin in scarlet, and on iron in a dull purple (**10**).

A clue to a possible role played by mordants in the living cell is suggested by the work of SHIBATA *et al.* (**17**). They state that organo-metallic complex compounds are formed between anthocyanin pigments and the salts of the alkaline earths and the heavy metals. They added metallic salts to solutions of certain extracted anthocyanin pigments. Such pigments extracted from Rosa Gallica turned red with HCl and acetic acid; violescent red with $CaCl_2$, $SrCl_2$, $MgCl_2$, and $MnCl_2$; light violescent red with $BaCl_2$ and $ZnCl_2$; dark violescent red with $Al_2(So_4)_3$, $Cr_2Cl_3$, and $NiCl_2$; red orange with $Cu(C_2H_3O_2)_2$ and $HgCl_2$; orange with NaOH and $(NH_4)_2MoO_4$; violet with $SnCl_2$ and $Fe_2Cl_6$; green with $Pb(C_3H_3O_2)_2$; yellow with $K_2CO_3$. In other words, the range extended from green to deep red, depending on the salt used.

SHIBATA and coworkers are of the opinion that blue anthocyanin pigments in plants are complex organo-metallic compounds, the pigments probably being coordinated with calcium or magnesium. The violet, violescent red, or red pigments are considered as analogous complex compounds containing fewer auxochrome hydroxyl groups or a mixture of the blue pigment complex and a red oxonium salt. The red oxonium salt is presumably formed with an excess of acid, the anion of the acid becoming attached to the pigment molecule.

CURREY (**1**) investigated bluing in roses. The variety Hadley was selected as the type which often blues and Lady Maureen Stewart as the one which seldom blues. He determined that the red color in the rose petals was produced by the

formation of a red oxonium salt between the pigment and the tannins and that the tannins were the only acids found in the cell sap. He noted that Lady Maureen Stewart contained 1.9 times more pigment and 2.0 times more tannin than Hadley. The former contained 1.39 times more tannin than pigment. The Hadley rose also contained 1.39 times more tannin than anthocyanin pigment. CURREY assumed that the tendency of Hadley to turn blue was due to the fact that it contained fewer tannins than the other rose. He disregarded the fact that Hadley also had a reduced amount of pigment directly proportional to the reduced amount of tannin. It would be just as logical to assume that bluing of Hadley is due to less pigment in the petals.

CURREY also considered that the ash analysis of the two roses showed no significant differences. By recalculating his figures, however, it is obvious that Lady Maureen Stewart had 0.9 as much silica as Hadley, 1.4 times more aluminum and iron, 1.3 times more lime, 1.1 times more magnesium, 0.8 as much sulphur, 1.3 times more phosphorus, 2.4 times more soda, and 1.3 times more potash. Aluminum and iron are both important mordant elements. Lady Maureen Stewart contained more of these elements. It is possible also that the lack of salts was a factor in the bluing of Hadley.

HALL (**5**) reports that in dyeing, tannic acid can unite with metallic salts to form insoluble compounds and that these have the power of retaining dyes. The compounds containing tannic acid, metal, and dyestuff are more nearly insoluble and stable than similar ones in which the metallic salts are absent. PERKIN and EVEREST (**15**) state that the great increase of the basicity of the oxygen atom of the pyrone ring that arises in passing from the flavonol pigment to the anthocyanin pigment is presumably the cause of the latter having the power of dyeing on tannins as well as basic mordants. It is possible that organo-metallic compounds that might be formed with anthocyanin pigments increase the molecular weight of the pigment and thereby influence color (**4**).

A number of investigators have attempted to maintain petal turgidity and brilliant color of cut flowers by placing the stems in various solutions. DUCOMET and FOURTON (**2**) found that the following aqueous solutions gave positive results: sugar and sodium chloride, sugar and dipotassium phosphate, sugar and chloral, sugar and manganese sulphate. LAURIE (**9**) indicated that combinations of hydrazene sulphate, copper sulphate, sucrose, sodium amytal, and metallic zinc powder in proper concentrations may reduce bacterial activity, increase transpiration, lower the rate of respiration, and provide the most suitable pH and osmotic concentration. KNUDSEN (**8**) and HITCHCOCK and ZIMMERMAN (**6**) placed the stems of cut flowers in aqueous solutions containing various chemicals and found no solution of any particular value. VOLZ (**21**) used a solution of aluminum sulphate in water.

There are available at present at least two commercial preparations[1] for use in aqueous solutions. Both are principally sugar and aluminum salts. The length of time during which cut roses remain turgid and of good color is considerably increased by the use of these solutions. After a few days the petals of red roses lose their original color, just as do those with the stems in water, but the former maintain highly satisfactory and pleasing color as contrasted with the dull violet or bluish tinges of the latter. The petals also maintain exceptional turgidity. There is apparently no clogging of the conducting tissues of the stems; at least it is never necessary to maintain petal turgidity by clipping off the ends of the stems. This indicates a rather unusual internal physiological condition. Physiologically, aluminum salts have been reported as increasing the permeability of protoplasm, thus making it more permeable to sugars, increasing the action of diastase, slowing down photosynthesis (**3**), causing protoplasm to set and harden (**18**), and inhibiting anthocyanin production (**7**).

Apparently the aluminum mordant salts and sugars complement each other. If either is eliminated from the commercially available solutions, color and turgidity are not so satisfactory. With these solutions the color of the developing inner petals is affected to a much greater extent than the matured outer ones, which tend to fade and blue during storage. The solutions would be more desirable if the color of the matured outer petals were preserved. An undesirable feature of storage of roses with the stems in water or an aqueous solution is the tendency of the buds to continue to open during storage.

NEFF (**12**) reported favorable results in regard to the preservation of color and keeping qualities of cut roses under conditions of light, low temperature, and the use of commercially available solutions and others containing potassium nitrate and sucrose.

## Experimental results

Red Hollywood rose buds stored at 32°–38° F. for any length of time have a tendency to lose the original color shadings and begin to blue. Storage at low temperature and in carbon-dioxide concentrations high enough to cause the buds to remain in a tight condition resulted in considerable tendency to blue. This was also true for roses stored at low temperature in an atmosphere containing added carbon dioxide and reduced oxygen.

Generally the petals of roses previously stored expanded rapidly after removal from storage. The petals were crinkly along the edges and seemed to lack substance. The flowers failed to remain as turgid or as well colored as freshly cut roses under similar conditions.

[1] U.S. patents 2168304 and 2230931.

### TURGOR AND LOW TEMPERATURE

The manner in which roses are handled previous to storage determines to a considerable degree their response under various conditions of storage. Fresh Hollywood roses that had never been placed in water were divided into groups of six each and exposed to the drying atmosphere of a room at 32°–38° F. for 3, 12, and 24 hours. After the drying treatment, the roses were placed under bell jars to protect them from further excessive loss of moisture. The stems were not in water at any time during the drying treatment or during the remaining storage period. An additional group of roses was left with the stems in water throughout the experiment.

The roses exposed for 3 hours lost 0.46 per cent, for 12 hours 0.7 per cent, and for 24 hours 2.7 per cent of their original weight. At the end of the 12-day storage period all roses stored with the stems out of water had lost an additional 1.7 per cent of their weight.

After 12 days in storage the flowers were removed to a room at 72° F., the stems placed in water and a comparison made with fresh roses. The roses were weighed when they were first brought into room temperature and after 12, 22, 31, and 43 hours. The fresh roses gained 9.5 per cent in weight in the first 12 hours at room temperature but lost weight by the twenty-second hour. By 38.5 hours they weighed 5.4 per cent less than the original weight. The roses exposed to 3 hours of drying gained 7.7 per cent in weight the first 12 hours, 8 per cent the next 19 hours, but by the forty-third hour weighed 1.2 per cent less than their original weight. The original weight refers to the weight of the roses when first placed in storage. The buds of the stored roses opened a little more rapidly than did the fresh buds. The individuals exposed to drying for 12 hours gained 4.1 per cent in weight the first 12 hours, 4.1 per cent the next 10 hours, 2.1 per cent the following 9 hours, but weighed 6 per cent less than the original weight by the forty-third hour. The buds opened a trifle more slowly than fresh ones. Those exposed to 24 hours of drying gained 0.6 per cent in weight the first 22 hours at room temperature. The following 9 hours they weighed 15.5 per cent less than their original weight. They opened much more slowly than the fresh buds and were only half opened at the time they wilted. Those stored with their stems in water lost weight at room temperature and the buds opened faster than fresh ones.

These results suggest turgor control as a method of maintaining rose buds in a tight condition during storage. Buds opened least during storage when exposed to the drying atmosphere for 24 hours before being placed under bell jars. Twenty-four hours of drying was apparently too long, as they failed to open properly when removed from storage and placed at room temperature with the stems in water.

None of the preceding lots retained their original color shadings during storage; all had a tendency to blue. When removed to room temperature none of the storage lots retained petal turgidity or color as well as did freshly cut roses.

### Low temperature and carbon dioxide

Rose buds were inclosed in large metal containers and placed in storage at 32°–38° F. The tops of the containers were made with grooves which were filled with water, into which the edge of the lids fitted. This arrangement provided a water seal, preventing excessive loss of carbon dioxide. A layer of water on the bottom of the containers maintained a reasonably high humidity. The buds were stored in the containers either with their stems in water or supported on wire racks out of water. The containers were fitted with two valves, through which carbon dioxide could be introduced. Carbon dioxide concentrations of 2, 5, 10, 15, and 20 per cent were maintained. After 12–20 days of storage the roses were inferior to freshly cut ones. The chief difficulty was their tendency to blue; the higher the carbon dioxide concentrations the greater was the tendency to do so. At concentrations of 2 and 5 per cent the buds did not remain as tightly closed as when higher concentrations were used. The roses stored with carbon dioxide were inferior to fresh ones when comparisons were made as to color and turgidity under ordinary room conditions.

Sweet peas were similarly stored with stems out of water at 32°–38° F. but without the use of carbon dioxide. High humidity in the individual chambers kept the petals fresh and turgid over a 16–21-day storage period. The specimens were removed from storage in excellent condition, with no petal drop. The stored flowers were turgid almost as long as fresh blooms when subsequently placed at room temperature with the stems in water, but they had lost all odor during storage.

### Carbon dioxide and reduced oxygen

Talisman rose buds were stored as previously stated in metal containers at 32°–38° F. for 5 days in atmospheres consisting of 9–10 per cent carbon dioxide and varied amounts of oxygen. To obtain the desired atmosphere, air was displaced from the metal containers by means of nitrogen gas until the desired level of oxygen was secured, then carbon dioxide was introduced. At the end of 5 days the buds stored in 4.4 and 6 per cent oxygen had opened very slightly; in 9 per cent oxygen they had opened noticeably; and in 11 per cent oxygen still more so. At the end of 5 days the roses were unintentionally frozen. This injured the stems somewhat, particularly directly below the calyx cup. The samples in 11 per cent oxygen had the least stem injury; it was progressively greater in 9 and 6 per cent, up to 4.4 per cent oxygen, where the greatest injury occurred.

### LOW TEMPERATURE, LIGHT, STEMS IN SOLUTIONS

Freshly cut buds of Hollywood roses were stored in three closed metal containers (A, B, and C) in a refrigerated room at 32°–38° F. or in two closed glass humidity chambers (D and E). High relative humidity was maintained by the layer of water on the bottom of each container. A sheet of window glass loosely placed over the top of the metal containers, in place of the metal cover, allowed for the admission of light as desired.

The buds were divided into groups of four previous to being placed in the containers. Container A received eight groups, B three groups, C two groups, humidity chambers D and E four groups. The lower portions of the stems were immersed in flasks containing Floralife, a commercial product, to which sucrose had been added at the rate of 60 gm. to a quart of solution.

Roses in container A received approximately 48 foot candles of Mazda light for an 18-day period. Container B received approximately 20 foot candles for an 18-day period. Container C was covered for the first 12 days so as to exclude light; during the last 6 days the cover was removed and the roses exposed to 10 foot candles of Mazda light. Roses in D (glass humidity cases) were illuminated by a 20-watt daylight-type fluorescent lamp. The flowers received approximately 60 foot candles of light at the surface of the petals. Container E was illuminated by Mazda light which was first passed through an aqueous $CuCl_2$ filter 2.2 cm. in thickness (1 gm. of $CuCl_2$ to 1000 ml. of water), resulting in approximately 26 foot candles of light at the surface of the petals. Light was supplied to D and E for the first 6 days of storage, then discontinued until the twelfth day, at which time illumination was resumed.

Preliminary work had indicated that if roses whose stems were in the solutions were taken from the refrigeration room from time to time it might be beneficial. Consequently groups 2 and 6 in container A, groups 1 and 3 in container B, and group 2 in container C were placed in the 72° F. room for 30-minute periods on the second, third, fourth, fifth, seventh, and eighth days of storage. Groups 3 and 7 from container A were subjected to two 30-minute periods at 72° F. on the second, third, fourth, and fifth days and one 30-minute period on the seventh and eighth days of storage. The second day of storage, groups 4 and 8 of container A were placed at 72° F. for 30 minutes for six consecutive transfers. The third and fourth days they were taken out of the storage temperature twice a day for 30-minute periods; the seventh and eighth days they were removed only once a day. Group 1 of containers A, B, C, D, and E were not removed from the storage temperature until the end of the experiment.

Roses in D and E were transferred to the 72° F. room for 30-minute periods the second day of storage in the case of groups 2, 3, and 4. On the fourteenth day of storage group 2 in case D and group 2 in case E were placed in the 72° F. room

for 30 minutes, and then at 33° F. for 30 minutes for four consecutive transfers. Groups 3 in D and E were given six consecutive transfers. Group 4 was given eight consecutive transfers of 30 minutes each.

Table 1 shows the keeping qualities of the roses after removal from storage to a room at 72° F. with the stems in water.

TABLE 1

CONDITIONS OF STORAGE AND KEEPING QUALITIES OF HOLLYWOOD ROSES AFTER REMOVAL FROM STORAGE* TO ROOM AT 72° F., WITH STEMS IN WATER. STORAGE TEMPERATURE APPROXIMATELY 32°–38° F.; HUMIDITY HIGH

| Container | Group | Days in storage | Hours with stems in solution | Hours in light | | Foot candles of light | | Times transferred | Number of turgid flowers per group per day after removal from storage to room at 72° F. (stems in water) Days | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Mazda | Fluorescent | Mazda | Fluorescent | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |
| A | 1 | 8 | 192 | 192 | 0 | 48 | 0 | 0 | 4 | 4 | 4 | 2 | ... | ... | ... | ... | ... |
| | 2 | 8 | 192 | 192 | 0 | 48 | 0 | 6 | 4 | 4 | 4 | ... | ... | ... | ... | ... | ... |
| | 3 | 8 | 192 | 192 | 0 | 48 | 0 | 10 | 4 | 4 | 4 | 3 | ... | ... | ... | ... | ... |
| | 4 | 8 | 192 | 192 | 0 | 48 | 0 | 12 | 4 | 4 | 4 | 4 | ... | ... | ... | ... | ... |
| | 5 | 18 | 432 | 432 | 0 | 48 | 0 | 0 | 4 | 4 | 1 | 1 | 1 | ... | ... | ... | ... |
| | 6 | 18 | 432 | 432 | 0 | 48 | 0 | 6 | 4 | 3 | 3 | 3 | 2 | 1 | ... | ... | ... |
| | 7 | 18 | 432 | 432 | 0 | 48 | 0 | 10 | 4 | 4 | 4 | 4 | 4 | 2 | ... | ... | ... |
| | 8 | 18 | 432 | 432 | 0 | 48 | 0 | 12 | 4 | 4 | 4 | 3 | 3 | ... | ... | ... | ... |
| B | 1 | 8 | 192 | 192 | 0 | 20 | 0 | 6 | 4 | 4 | 4 | 2 | ... | ... | ... | ... | ... |
| | 2 | 18 | 432 | 432 | 0 | 20 | 0 | 0 | 4 | 4 | 4 | 4 | 4 | 3 | 2 | ... | ... |
| | 3 | 18 | 432 | 432 | 0 | 20 | 0 | 6 | 4 | 4 | 3 | 3 | 2 | 1 | ... | ... | ... |
| C | 1 | 18 | 432 | 144 | 0 | 10 | 0 | 0 | 4 | 4 | 4 | 4 | 3 | 1 | ... | ... | ... |
| | 2 | 18 | 432 | 144 | 0 | 10 | 0 | 6 | 4 | 4 | 4 | 2 | 1 | 1 | ... | ... | ... |
| D | 1 | 14 | 336 | 0 | 192 | 0 | 60 | 0 | 4 | 4 | 4 | 4 | 3 | 2 | ... | ... | ... |
| | 2 | 14 | 336 | 0 | 192 | 0 | 60 | 5 | 4 | 4 | 4 | 4 | 2 | 2 | ... | ... | ... |
| | 3 | 14 | 336 | 0 | 192 | 0 | 60 | 7 | 4 | 4 | 4 | 4 | 4 | 3 | 2 | ... | ... |
| | 4 | 14 | 336 | 0 | 192 | 0 | 60 | 9 | 4 | 4 | 4 | 4 | 4 | 3 | 2 | ... | ... |
| E | 1 | 14 | 336 | 192† | 0 | 20 † | 0 | 0 | 4 | 4 | 4 | 4 | 3 | 2 | ... | ... | ... |
| | 2 | 14 | 336 | 192† | 0 | 20 † | 0 | 5 | 4 | 4 | 4 | 4 | 3 | 3 | ... | ... | ... |
| | 3 | 14 | 336 | 192† | 0 | 20 † | 0 | 7 | 4 | 4 | 4 | 4 | 4 | 4 | 2 | 1 | 1 |
| | 4 | 14 | 336 | 192† | 0 | 20 † | 0 | 9 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 1 | ... |

* Fresh buds with stems in water and under room conditions remained turgid 3–4 days.
† Filtered light.

As indicated by table 1, the storage roses may remain turgid longer than fresh ones at ordinary room temperature, and those stored 18 days may remain turgid longer than those stored 8 days. HITCHCOCK and ZIMMERMAN (6) stated that flowers stored at 5° C. for 7 days or longer may wilt much sooner when removed to temperatures above 22° C. than fresh flowers. It evidently takes a certain amount of time in storage before the effect of the conditions become permanent after the flowers are removed to room temperature and the stems placed in water. This permanent effect on turgidity of petals seems to be correlated with the state

of development of the buds. Rose buds tend to open, even under conditions of low temperature, especially if they are in water or a water solution. By the fifth to seventh day of storage this tendency becomes rather marked; up to that time the buds remain relatively tight. The roses removed after 8 days of storage were one-third or less open and so had not developed sufficiently for the storage treatment to have a permanent effect on the turgidity of the petals. On the other hand, at the end of the 18-day storage period the roses were one-half to two-thirds open, so were sufficiently advanced for the storage treatment to have a permanent effect on petal turgidity.

Table 1 indicates that removing roses to room temperature for short periods of time during storage may help to maintain petal turgidity after the flowers are finally removed and placed under ordinary room conditions with the stems in water. This may be true for roses stored 18 days and receiving 48 foot candles of Mazda light, or filtered Mazda light of 20 foot candles, but not for roses receiving 48 foot candles of Mazda light for only 8 days or 20 foot candles for 18 days.

One of the effects of shifts in temperature during storage is the appearance of drops of sweet liquid which exude from stems and the veins of the leaves. Another effect is the appearance of brown spots on the petals, apparently caused by the high concentration of sugar. The roses exposed to fluorescent light showed considerably less exudation than those stored under other types of light. At the time of removal from storage there were no brown spots on the petals, when it was used. After a few hours at room temperature, however, the brown spots appeared. Another result from the use of fluorescent light is that the petals opened in a normal manner after removal to room temperature. After storage under Mazda light the petals were crinkly and definitely not normal in their subsequent development at room temperature.

## Solutions

Several mixtures were developed that were successful when used in solution in maintaining petal color and petal turgidity. The solutions are basically water, sucrose, and various metallic mordants. Tables 2 and 3 list the proportions of salts and sucrose used per 50 ml. of tap water, a solution sufficient for one rose. Greatly increasing the amount of solution used per rose may result in excessive injury to the stems or petals. Better Times roses were used in these experiments.

The following sugar solutions with single salts added produced indifferent results: lead acetate, zinc sulphate, aluminum acetate, aluminum chloride, aluminum oxide, ferrous oxalate, ferrous phosphate, lead chromate, and ferrous ammonium sulphate. A sugar solution with potassium dichromate and oxalic acid and a solution composed of sugar with tannic acid and acetic acid gave only fair results.

The following sugar solutions with two salts were used with varied results. A stock solution sufficient for one rose was made up of 0.02 gm. of ferrous sulphate, 1.25 gm. of sucrose, and 50 ml. of tap water. To this basic solution was added 0.02 gm. of one of the following salts: cadmium chloride, silver sulphate, cobalt

TABLE 2

EFFECT OF SOLUTIONS* ON COMPARATIVE KEEPING QUALITIES OF FRESHLY CUT BETTER TIMES ROSES UNDER ROOM CONDITIONS; 1.5 GM. SUGAR ADDED PER ROSE

| Salt per rose | Ferrous chloride | | Ferrous sulphate | | Chromium sulphate | | Ceric sulphate | | Stannous chloride | | Stannous sulphate | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Petals turgid† (days) | Original pH | Petals turgid (days) | Original pH | Petals turgid (days) | Original pH | Petals turgid (days) | Original pH | Petals turgid (days) | Original pH | Petals turgid (days) | Original pH |
| 0.015 | ..... | ..... | 4 | 5.6 | 6 | 5.4 | 4 | 4.6 | 5 | 3.9 | ..... | ..... |
| 0.02 | 6 | 4.9 | 6 | 5.5 | 6 | 4.5 | 5–6 | 3.5 | ..... | ..... | ..... | ..... |
| 0.025 | 5 | 4.7 | 6–7 | 5.1 | 4 | 4.2 | 5–6 | 3.3 | ..... | ..... | 6 | 2.8 |
| 0.03 | ..... | ..... | 6–7 | 5.0 | 4 | 4.0 | 5–6 | 3.0 | ..... | ..... | ..... | ..... |
| 0.035 | ..... | ..... | 6 | 4.8 | 4 | 3.8 | 5–6 | 2.9 | ..... | ..... | ..... | ..... |

* Similar buds with stems in water and under room conditions remained turgid 3–4 days.
† Or until they dropped.

TABLE 3

EFFECT OF SOLUTIONS* ON COMPARATIVE KEEPING QUALITIES OF FRESHLY CUT BETTER TIMES ROSES

| Salt used | Sugar per rose (gm.) | Salt per rose (gm.) | Original pH | Petals turgid (days) | pH of solutions determined twice daily | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Second day | | Third day | | Fourth day | | Fifth day | | Sixth day | |
| | | | | | A.M. | P.M. | A.M. | P.M. | A.M. | P.M. | A.M. | P.M. | A.M. | P.M. |
| Ferrous chloride | 1.25 | 0.02 | 5.2 | 6 | 5.2 | 4.7 | 4.3 | 4.1† | 4.9 | 4.7 | 4.7 | 4.8 | 4.7 | 4.7 |
| Ferrous sulphate | 1.25 | 0.03 | 5.0 | 5–6 | 4.8 | 4.3 | 4.3 | 3.9† | 4.9 | 4.4 | 4.6 | 4.6 | 4.5 | 4.4 |
| Ferrous chloride | 0.125 | 0.02 | 5.2 | 6 | 5.0 | 4.5 | 4.1 | 3.8† | 5.2 | 4.9 | 5.0 | 5.1 | 5.0 | 4.9 |
| Ferrous sulphate | 0.125 | 0.03 | 5.1 | 5 | 5.2 | 4.6 | 4.2 | 4.0† | 5.0 | 4.7 | 4.8 | 5.0 | 4.7 | 4.6 |
| Ferrous chloride | 0.25 | 0.02 | 5.2 | 6–7 | 5.4 | 5.1 | 4.5 | 4.2† | 4.6 | 4.5 | 4.5 | 4.5 | 4.7 | 4.7 |
| Ferrous sulphate | 0.25 | 0.03 | 5.1 | 6–7 | 5.3 | 4.7 | 4.4 | 4.2† | 5.0 | 4.7 | 4.8 | 4.9 | 4.7 | 4.6 |

* Fresh buds with stems in water and under room conditions remained turgid 3–4 days.
† Tap water added to each container, replacing water lost through evaporation and transpiration.

sulphate, cobalt chloride, sodium tungstate, uranium chloride, antimony chloride, antimony sulphate, bismuth acetate, molybdic acid, copper sulphate, ferric chloride, nickel chloride, nickel sulphate, lead chloride, manganese chloride, and mercurous chloride. Most of the solutions proved too concentrated, as shown by the

excessive burning of the stem or wilting of the petals. The use of molybdic acid, cobalt, bismuth, lead, uranium, and tin salts appeared to have promise.

## Summary

1. Rose buds may be maintained in the desired state of maturity during storage at low temperature by avoiding placing the stems in water or aqueous solution during such period. The desired state of maturity also may be maintained by storing the roses in an atmosphere containing added carbon dioxide, or carbon dioxide and a reduced amount of oxygen.

2. Rose buds have a strong tendency to open during storage. This is particularly true when stored with their stems in water or aqueous solutions.

3. Roses develop objectionable color characteristics under low temperature storage. These tendencies seem to increase when stored in an atmosphere containing large amounts of carbon dioxide, or carbon dioxide and a reduced amount of oxygen.

4. As a rule, roses which have been stored do not maintain satisfactory petal turgidity or petal color after removal from storage to room temperature with their stems in water.

5. Roses which have been stored with the stems in a sugar-mordant solution under proper conditions of light and temperature and for a sufficient length of time may remain turgid longer than fresh roses when placed in water at room temperature.

6. Solutions containing water, sucrose, and a salt of certain heavy metals may materially aid in maintaining petal color and turgidity of cut roses.

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## LITERATURE CITED

1. CURREY, G. S., The cause of blueing in red roses. Jour. Roy. Soc. New So. Wales **61**:307–314. 1927.
2. DUCOMET, V., and FOURTON, L., Preservation of cut flowers. Florists Exch. **31**:597–598. 1911.
3. FLURI, M., Der einfluss von Aluminumsalzen auf das Protoplasma. Flora **99**:81–126. 1909.
4. GEORGIEVICS, G. VON, and GRANDMOUGIN, E., The text-book of dye chemistry. Transl. and revised from 4th German ed. by A. Mason. London. 1920.
5. HALL, A. J., Dyes and their application to textile fabrics. London. 1923.
6. HITCHCOCK, A. E., and ZIMMERMAN, P. W., Effects of chemicals, temperature, and humidity on the lasting of cut flowers. Contr. Boyce Thompson Inst. **2**:196–203. 1930.
7. KATIC, D. L., Beitrag zur Kenntnis der Bildung des roten Farbstoffs in vegetativen Organen der Phanerogamen. Inaugral-Dissertation Halle a.S., 1905. (Cited from WHELDALE, M., The anthocyanin pigments of plants. 1916.)
8. KNUDSEN, L., Preserving cut flowers. Amer. Flor. **43**:649–650. 1914.

9. LAURIE, A., Studies of the keeping qualities of cut flowers. Amer. Soc. Hort. Sci. **33**:595–597. 1936.
10. MATTHEWS, J., Application of dyestuffs. New York. 1920.
11. NEFF, M. S., Problems in the storage of cut carnations. Plant Physiol. **14**:271–284. 1939.
12. ———, Color and keeping qualities of cut flowers. BOT. GAZ. **101**:501–504. 1939.
13. NEFF, M. S., and LOOMIS, W. E., Storage of French marigolds. Proc. Amer. Soc. Hort. Sci. **33**:638–685. 1936.
14. PERRET, A., Le froid en horticulture. Rev. Sci. Paris **5**:170–174. 1907.
15. PERKIN, A. G., and EVEREST, A. E., Natural colouring matters. London. 1918.
16. PERKIN, W. H., and KIPPING, F. S., Organic chemistry. Philadelphia. 1911.
17. SHIBATA, A. G., SHIBATA, Y., and KASIWAGI, I., Studies on anthocyanins: Color variations in anthocyanin. Jour. Amer. Chem. Soc. **41**:208–220. 1919.
18. SZÜCS, J., Über einnige charakteristische Wirkungen des Aluminumions auf das Protoplasma. Jahrb. wiss. Bot. **52**:269–332. 1913.
19. THORNTON, N. C., The use of carbon dioxide for prolonging the life of cut flowers, with special reference to roses. Contr. Boyce Thompson Inst. **2**:535–547. 1930.
20. ———, Changes in flower color as evidence of the effectiveness of carbon dioxide in reducing the acidity of plant tissue. Contr. Boyce Thompson Inst. **6**:403–405. 1934.
21. VOLZ, E. C., Studies on the keeping qualities of cut flowers. Unpublished thesis, Cornell University. 1918.
22. WEISER, H. B., Inorganic colloid chemistry. Vol. II. The hydrous oxides and hydroxides. New York. 1935.

# AUXIN STORAGE AS RELATED TO ENDOSPERM TYPE IN MAIZE

GEORGE S. AVERY, JR., JULIUS BERGER, AND BARBARA SHALUCHA

The extent to which auxin storage is related to kind of tissue is as yet unknown. Maize endosperms provide an excellent source of material to investigate this point; first, because a method exists (1) for the total extraction of auxin from them, and second, because they provide ontogenetically identical tissues which may differ genetically, the latter leading to the storage of different kinds and proportions of materials. Five well-known endosperm types were chosen for this investigation: brittle, waxy, floury, sugary, and two kinds of flint.

The first extraction method used involved successive washes with water. Embryos and endosperms were carefully separated, and the latter finely ground in a glass mortar. A 1-gm. sample of this ground material was placed on an asbestos filter and given twenty successive washes, each with 10 cc. of water (at 2-minute intervals). The aqueous filtrates from these twenty washes were collected in one flask; these and all subsequent samples were assayed by the deseeded *Avena* method. The twenty-first wash was collected and assayed separately in order to determine whether most of the auxin had been removed in the original washes. In no instance did the twenty-first wash contain as much as half of 1 per cent of the auxin extractable by this method. This experiment was repeated six times for each endosperm type, with good agreement, and the averages of these data are presented in table 1 (method A).

After these experiments were completed, an extraction method was developed (1) which makes it possible to distinguish between the relative amounts of free auxin and auxin precursor present in a given sample of maize endosperm. The so-called precursor is an as-yet unidentified compound which upon alkaline hydrolysis yields auxin.

For determining the free auxin content of the different endosperm types by the newer method, ground tissue was prepared as described, and 0.25-gm. samples were allowed to stand in 25 cc. of distilled water at 25° C. for 10 minutes. This preparation was centrifuged and the clear extract diluted to suitable concentrations for obtaining proportionality curvatures in the assay. The yields of free auxin (method B) from the different endosperm types are also presented in table 1.

Determinations of total auxin were made by heating 0.25-gm. samples of ground endosperm in borate buffer at pH 9.6 and 120° C. for 15 minutes. The hydrolyzed samples were neutralized, centrifuged, and dilutions made up for assay. The total

auxin yields thus obtained are included in table 1 (method C). The auxin precursor consists of the total auxin present after hydrolysis, minus the free auxin.

It may be noted from the table that the yields as determined by method B agree in a general way with those obtained by method A, that is, both methods give free auxin, but method B is much simpler. The free auxin content is of the same order for brittle, waxy, floury, and Canada flint, twice as high for sugary, and half as high for pop corn. Auxin precursor yield is from six to sixteen times that of the free auxin. It is of about the same magnitude in brittle, waxy, floury, and Canada

TABLE 1

AUXIN YIELDS FROM DORMANT MAIZE ENDOSPERMS OF DIFFERENT TYPES (EMBRYOS REMOVED)

| Endosperm type* | Auxin yields in millions of tdc per gram dry weight (or tens of micrograms of indoleacetic acid) | | | |
|---|---|---|---|---|
| | Method A | Method B | Method C | |
| | Free auxin (21 washes with water) | Free auxin (1 water extraction, 10 min. at 25° C.) | Total auxin (water suspension autoclaved at pH 9.6 and 120° C. for 15 min.) | Auxin precursor (total auxin minus free auxin) |
| Brittle | 0.56 | 0.71 | 5.2 | 4.5 |
| Waxy | 0.64 | 0.45 | 5.9 | 5.4 |
| Floury | 0.54 | 0.58 | 4.5 | 3.9 |
| Sugary | 1.5 | 1.3 | 13.2 | 11.9 |
| Flint (pop corn) | 0.26 | 0.20 | 3.2 | 3.0 |
| Flint (Canada flint) | 0.45 | 0.50 | 6.0 | 5.5 |

* First four samples obtained through the courtesy of the Department of Plant Breeding, N.Y. State College of Agriculture, Cornell University.

flint. In common with free auxin, it is somewhat lower in pop corn endosperm and markedly higher in sugary.

Thus of all the endosperm types tested, sugar corn endosperm was found to be the richest in both auxin and auxin precursor.

Auxin assays of four commercial varieties of sugar corn gave the results reported in table 2. Of these, the variety Country Gentleman possessed the highest total auxin (free auxin plus auxin precursor).

Auxin assays on immature corn kernels in various stages of development showed young kernels much richer in auxin than mature dormant ones. Such immature kernels are available at all times in commercial, dry-pack canned and frozen corn. Tests were therefore made on "Niblets" (Minnesota Valley Canning Company) and frozen cut Golden Bantam corn (Frosted Foods). Ten endosperms from each

sample were ground, heated in borate buffer at 120° C. and pH 9.6 for 15 minutes, neutralized, centrifuged, and the clear extract diluted for assay. Total auxin content of the Niblet endosperms was 32 million TDC per gram dry weight (equivalent to 320 micrograms of indoleacetic acid) and of the frozen endosperms 34 million TDC per gram. The former had more than 3 million TDC free auxin per gram dry weight, the latter only 0.9 million.

In view of the facts that indoleacetic acid has been isolated from corn (2) and that the auxin derived from the precursor is alkali stable (1), it is probable that 90 per cent or more of the auxin obtainable from corn is indoleacetic acid. The total auxin yields reported for dry-pack canned and frozen corn (immature stages of

TABLE 2

TOTAL AUXIN PRESENT IN DORMANT KERNELS OF FOUR VARIETIES OF SUGAR CORN*

| VARIETY | AUXIN† |
|---|---|
| Bantam Evergreen no. 36010 | 9.0 |
| Stowell's Evergreen no. 26172 | 11.4 |
| Country Gentlemen no. 38666 | 14.2 |
| Early Evergreen no. 26045 | 8.4 |

* Samples obtained through the courtesy of the Associated Seed Growers, Inc., Milford, Connecticut.

† In millions of TDC per gram dry weight of entire kernels (or in tens of micrograms of indoleacetic acid).

sugar corn) are the equivalent of more than 300 mg. of indoleacetic acid per kilogram dry weight.

It is clear that kernels of sugar corn are much richer in auxin than those with brittle, waxy, floury, or flint endosperm. So far as can be determined, these are the first assays demonstrating markedly different auxin storage in ontogenetically identical tissues from different varieties of the same species.

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## LITERATURE CITED

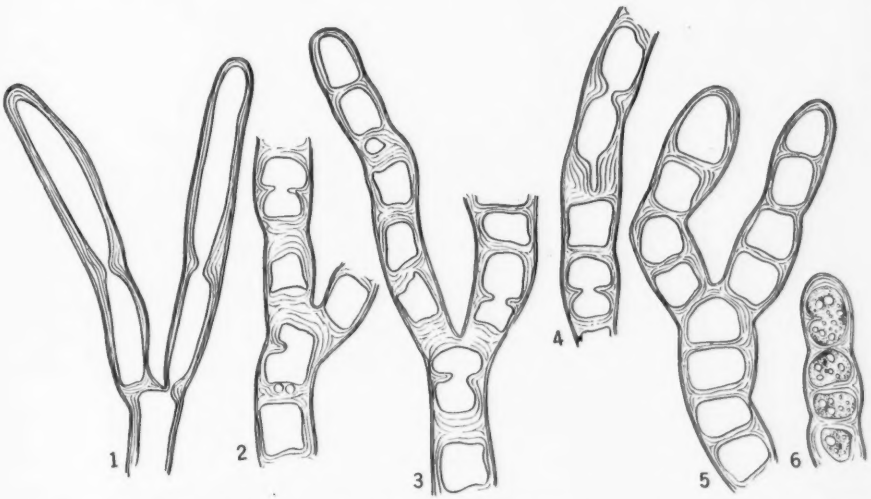
1. AVERY, G. S., JR., BERGER, J., and SHALUCHA, B., The total extraction of free auxin and auxin precursor from plant tissue. Amer. Jour. Bot. **28**:596–607. 1941.
2. HAAGEN SMIT, A. J., LEECH, W. D., and BERGREN, W. R., Estimation, isolation and identification of auxins in plant material. Science **93**:624–625. 1941.

# AKINETE FORMATION IN VAUCHERIA GEMINATA

M. S. RANDHAWA

(WITH EIGHT FIGURES)

An interesting variety of *Vaucheria geminata* (Vauch.) DC, probably var. *longistipitata* Chapman 1934, was collected by the writer in December, 1939, from the Chenab Gardens, Chak no. 45 G.B., near Gojra, District Lyallpur, Punjab, growing in the shade by the side of a water course. On examination it was found to be so extensively segmented that it hardly appeared like a *Vaucheria* at all. This




FIGS. 1–6.—Fig. 1, primary septation into coenocytes. Figs. 2–4, advanced stages in akinete formation. Fig. 5, mature akinetes. Fig. 6, same showing contents.

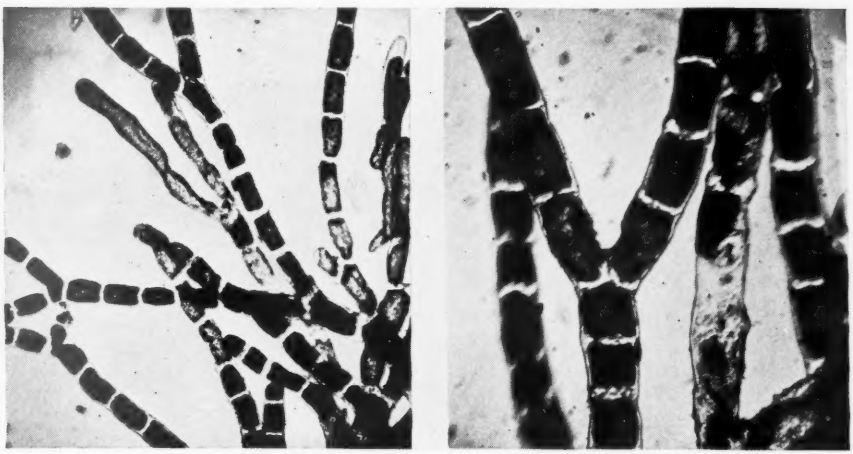
alga was again collected in December, 1940, and the same extensive akinete formation was found as in the previous year.

The purely vegetative filaments are 30–51 $\mu$ in diameter and display extensive dichotomous branching, the branching being radially arranged. Sex organs are very rarely formed and are borne at the ends of comparatively long branches, as in CHAPMAN's *longistipitata* variety of *V. geminata* described from Urbana, Ohio (1).

The interest of this form lies in its extensive akinete formation. This mode of propagation is so common in this alga that it has practically displaced the sexual

mode of propagation, which is found only as a relic sufficient to establish its identity.

A primary septation of the filament takes place by the thickening of the cell wall, on account of the apposition of mucilaginous material at particular points on both sides (fig. 1). These thickening bands are deposited radially, and protoplasm on both sides of the septa connects by means of narrow openings (fig. 2). This condition has a superficial resemblance to that sometimes obtained in *Sphaeroplea africana* Fritsch, where, however, the septa are composed of a number of radial processes, while in this alga they are comparatively homogeneous. The septa grow



FIGS. 7, 8.—Fig. 7 (left), general habit and early stages in akinete formation. Fig. 8 (right), parts of filaments showing akinetes.

radially and centripetally, ultimately meeting in the middle and fusing. Thus the filaments segment into a number of coenocytes, as in *Cladophora glomerata*. This process of the ingrowth of the cell wall is repeated until the coenocyte is subdivided into a number of akinetes (figs. 5, 8). Coenocytes constricted in the middle, somewhat dumbbell-shaped, may commonly be found (figs. 2–4). Sometimes ingrowth of cell wall may take place from one side only (figs. 2, 6). In this process of segmentation, islands of protoplasm may be left surrounded by thick mucilaginous material of the septa (fig. 3). Narrow projections of protoplasm, connected with the parent coenocyte on one side and recently cut off from a daughter akinete, may also be seen (fig. 4). Mature akinetes, more or less rectangular in outline, are rich in oil content and other food reserves, and sometimes have a more or less frothy appearance. They are 15–33 $\mu$ long and 30–50 $\mu$ broad. The contents stain

deep blue with Nile blue. It has not been possible to trace the ultimate fate of the akinetes and the mode of their liberation.

Akinete formation in *Vaucheria geminata* has been described by STAHL (3). His figures show the so-called cysts widely separated by more or less transparent septa, which is hardly the case with the Indian form. Like STAHL's alga, here also septation was noticed in a terrestrial alga, which was exposed to the risk of complete desiccation in a short time on account of the periodic closing of the canal water supply in the water course. The thick mucilaginous walls insure the alga against the risk of sudden desiccation, and the septation provides a more prolific and efficient method of propagation, almost completely substituting for a comparatively complicated mode of sexual propagation.

As already observed, the dichotomously divided, richly segmented, and radially arranged branches of this alga hardly appear like those of a *Vaucheria* (fig. 7). In its general appearance and mode of septation, resemblances to *Cladophora* are obvious. That the coenocytic condition of Siphonales is derived from a septate condition appears plausible. The reappearance of a septate condition under unfavorable environmental conditions may be regarded as an atavistic feature. As in *Cladophora*, the formation of septa in this alga is not related in any way with nuclear division. OLTMANN's (2) view that the Siphonales, particularly the Vaucheriaceae, are derived from septate forms like *Cladophora* appears to be considerably strengthened from these observations on the mode of septation in this alga.

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## LITERATURE CITED

1. CHAPMAN, F. B., The algae of the Urbana, Ohio, raised bog. Ohio Jour. Sci. **34**:327–332. 1934.
2. OLTMANN, F., Morphologie und Biologie der Algen. I. 2d ed. Jena. 1922.
3. STAHL, E., Ueber die Ruhezustände der *Vaucheria geminata*. Bot. Zeitschr. **37**:129–137. 1879.

# CURRENT LITERATURE

*Biological Symposia.* Vol. VI. Edited by JAQUES CATTELL. Lancaster, Pa.: Jaques Cattell Press, 1942. Pp. xii+355. Illustrated.

This sixth volume in the series of biological symposia is under the editorship of TH. DOBZHANSKY. The subject matter is presented in three sections.

I. *Symposium on temperature:* Introduction by H. H. PLOUGH; Temperature and spontaneous mutation, by H. H. PLOUGH; Induction of polyploidy in animals by extremes of temperature, by G. FANKHAUSER; Temperature and the differentiation of characters in *Drosophila*, by G. P. CHILD; Temperature factors in the development and evolution of sex, by EMIL WITSCHI; Isolating mechanisms, evolution, and temperature, by H. J. MULLER; Form and function in frizzle fowl: the interaction of hereditary potentialities and environmental temperature, by WALTER LANDAUER; Seasonal factors in gall wasp distribution, by A. C. KINSEY; and Role of temperature in the speciation of frogs, by J. A. MOORE.

II. *Symposium on isolating mechanisms:* Role of isolation in the differentiation of plant species, by G. L. STEBBINS, JR.; Isolating mechanisms in a complex of four toad species, by A. P. BLAIR; Isolating mechanisms in gall wasps, by A. C. KINSEY; and Isolating mechanisms in the genus *Drosophila*, by J. T. PATTERSON.

III. *Symposium on genetic control of embryonic development:* Role of genetic differentials in the embryonic development of amphibia, by V. C. TWITTY; Morphogenesis of genetic abnormalities in the chick, by V. HAMBURGER; and Physiological genetics of melanin pigmentation of the guinea pig, by SEWELL WRIGHT.

The papers show an increasing realization among biologists that, no matter with what material and with the aid of what tools they may work, they are seeking an understanding and a control of life processes. These symposia are contributing greatly toward the attainment of these aims.—J. M. BEAL.

*A Symposium on Respiratory Enzymes.* Madison: University of Wisconsin Press, 1942. Pp. xii+281. $3.00.

The book contains the addresses given at the symposium held co-operatively by the University of Wisconsin and the University of Chicago in September, 1941. The subjects discussed are intermediate carbohydrate metabolism; oxidative mechanisms in animal tissues; hydrogen transport; Pasteur effect; oxidases, peroxidases, and catalase; nicotinamide nucleotide enzymes; flavoproteins; cytochromes; phosphorylation of carbohydrates; metabolic cycles and decarboxylation; transamination; tumor, bacterial, and animal tissue respiration.

The book is an excellent summary of what is probably the most active phase of enzyme chemistry. No attempt is made to treat each enzyme exhaustively. Rather, the plan is to give recent developments, to interpret these in relation to past findings, and to call attention to unsolved problems. The structure, mechanism of the action, and properties of the enzymes are emphasized. An interesting feature is several pages of informal photographs of those attending the symposium, including a number of the participants.—S. V. EATON.

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